

" Patent  
Attorney Docket No. 0155.130US  
(formerly 18097-030310US)  
BDSM Docket No. 032705-006

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

03 OCT -8 PH 7:05

**REQUEST BY APPLICANTS FOR INTERFERENCE  
PURSUANT TO 37 C.F.R. § 1.607**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

By the present submission, Applicants request that an interference be declared between the instant application to Punnonen *et al.* ("Punnonen") and U.S. Patent No. 6,479,258 ("the '258 patent") issued to Short.

A Reply and Amendment to the outstanding Office Action mailed by the U.S. Patent and Trademark Office on June 25, 2003 (Paper No. 15) was filed on August 25, 2003.

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**R E M A R K S**

Applicants present a Request By Applicants For Interference Pursuant to 37 CFR § 1.607, wherein Applicants respectfully request that an interference be declared between the above-referenced application and the '258 patent issued to Short. The information required by 37 CFR § 1.607(a) is set forth under headings that correspond to the subsections of § 1.607 to facilitate consideration by the Examiner.

Specific support for each element of the claims can be found in **Appendix A**, attached hereto. A copy of the '258 patent, which issued from U.S. Application Serial No. 09/495,052, filed January 31, 2000 ("the Short '052 application"), is submitted herewith for the Examiner's convenience.

**REQUEST FOR INTERFERENCE**

**I. IDENTIFICATION OF THE PATENT WHICH INCLUDES SUBJECT MATTER WHICH INTERFERES WITH THE '869 APPLICATION**

The patent which claims subject matter which interferes with subject matter claimed in the present application ("the '869 application") is U.S. Patent No. 6,479,258 ("the '258 patent") issued on November 12, 2002 to Jay M. Short for "Non-Stochastic Generation of Genetic Vaccines".

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The '258 patent was issued on U.S. Application Serial No. 09/495,052, filed January 31, 2000 ("the Short '052 application"), which purports on its face to be a continuation-in-part (CIP) of U.S. Application Serial No. 09/276,860, filed March 26, 1999 ("the '860 application"), which is a continuation-in-part of U.S. Application Serial No. 09/246,178, filed February 4, 1999 ("the '178 application"), now U.S. Patent No. 6,171,820 ("the '820 patent"), which is a continuation-in-part of U.S. Application Serial No. 09/185,373, filed November 3, 1998 ("the '373 application"), which is a continuation-in-part of U.S. Application Serial No. 08/760,489 ("the '489 application"), filed December 5, 1996, now U.S. Patent No. 5,830,696 ("the '696 patent"), which claims benefit of U.S. Provisional Application No. 60/008,311 filed Dec. 7, 1995 ("the '311 provisional").<sup>1</sup> Diversa Corporation is the assignee named on the face of the patent.

## II. PRESENTATION OF A PROPOSED COUNT

Attached **Appendix B** sets forth the proposed Count. The proposed Count is an alternative Count prepared after consideration of the subject matter claimed by the respective parties. As required by 37 CFR § 1.601(f), the proposed Count "defines the interfering subject matter between . . . one or more applications and one or more patents."

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<sup>1</sup> The priority claimed on the face of the '258 patent differs from what is set forth in the file history of the '258 patent. *See Appendix F* for a depiction comparing the various priorities claimed in the '258 patent compared to what is seen on the face of the patent.

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The Count is proposed in alternative form because of the slightly different language utilized by the respective parties to describe the same invention. The interfering subject matter between Punnonen and Short relates to methods for obtaining immunomodulatory polynucleotides. The proposed Count comprises Claim 30 of the '258 patent and Claim 47 of the instant application set forth in the alternative.

**III. IDENTIFICATION OF THE CLAIMS OF THE '258 PATENT WHICH CORRESPOND TO THE PROPOSED COUNT**

Claims 1-86 of the '258 patent are believed to correspond to the proposed Count. Claims 1, 3, 5, 53, 59, 60 and 61 are independent claims. Claim 1 of the '258 patent is directed to a method of obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization.

**IV. CLAIMS OF THE '869 APPLICATION WHICH CORRESPOND TO THE PROPOSED COUNT**

Claims 1-46 were previously canceled without prejudice or disclaimer as to the subject matter contained therein, and Claim 47 was added in the Amendment and Reply dated March 27, 2003. New Claim 47 is essentially copied from Claim 30 of the '258 patent. Claim 47 is one alternative of the proposed Count. To assist the Examiner in this regard, Applicants attach

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**Appendices A, C, D and E.** **Appendix A** is a chart providing an element-by-element recitation of Claim 47 of the '869 application, and an indication of the passages in the '869 application<sup>2</sup> where, at the very least, the Claim finds support. **Appendix D** is a chart providing a side-by-side comparison of Claim 47 of the '869 application with the proposed Count, which is provided in **Appendix B**. **Appendix D** explains the rationale for including this claim in the interference based on the proposed Count. Applicants also provide **Appendix C**, which is a chart providing a side-by-side comparison of the Claims of the '258 patent with the proposed Count. Copies of the references cited in **Appendix C** and listed in **Appendix G** are being jointly submitted with an Information Disclosure Statement. **Appendix C** explains the rationale for including these claims in the interference based on the proposed Count. **Appendix E** is a diagram which illustrates the dependencies of the '258 patent claims for use in combination with **Appendix C**.

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<sup>2</sup> The present '869 application was filed on November 28, 2000. The '869 application is a continuation of U.S. Serial No. 09/248,716, filed February 10, 1999, which claims benefit of Provisional Application Serial No. 60/074,294, filed February 11, 1998. The '258 patent was substantially copied from at least four PCT publications of the assignee of the present '869 application ("Maxygen"), including PCT/US99/03020 (WO 99/41368), which corresponds to the disclosure of the present '869 application, PCT/US99/03023 (WO 99/41402), PCT/US99/02944 (WO 99/41383), and PCT/US99/03022 (WO 99/41369), all of which were published August 19, 1999. Accordingly, Short can only be accorded benefit of its January 31, 2000 filing date of its U.S. Application Serial No. 09/495,052 ("the Short '052 application"), which is the first Short filing after the publications by Maxygen, and is the first application in which Short provided written description support (copied from Maxygen's applications) for the claims in the '258 patent. As such, Punnonen should be designated Senior Party in the interference.

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**A. Brief Summary of the '258 Patent Claim Relationships**

As described in **Appendix C** and illustrated in **Appendix E** hereto, Claim 30 of the '258 patent (highlighted in red and with a box in an original or by a box in a copy) is one alternative of the Count. Claim 30 is dependent on, and thus includes the recitations of, Claim 29 and Claims 1-6. As such, Claim 30 anticipates Claims 29 and Claims 1-6, and thus those claims are appropriately designated as corresponding to the Count. All of Claims 7-52 and 70-86 depend ultimately from one or more of Claims 1-6, and contain no recitations which render them patentably distinct (as discussed more fully below) from such claims. Therefore, Claims 7-52 and 70-86 are appropriately designated as corresponding to the Count.

In addition to Claims 1, 3, 5, 53, 59, 60 and 61 are independent claims. While Claims 53, 59, 60 and 61 do not depend from Claims 1-6, Claims 53 and 59-61 are obvious over one or more of Claims 1-6 (as discussed more fully below). Claims 54-58, 62-66, and 67-69 depend ultimately from Claims 53, 59-61 or any of Claims 1, 3, 5, 53 and 59-61, respectively. Therefore, Claims 53-69 are also appropriately designated as corresponding to the Count.

**Appendix C** describes in detail the reasons that all of the claims of the '258 patent correspond to the Count, including examples of references to documents that were publicly available.

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**B. Claims which are dependent on Claim 1**

Claims 2, 27-28, and 70-84 depend ultimately on Claim 1, which is anticipated by the Count (as is Claim 2). Claims 27 and 28 merely recite that the optimized recombinant polynucleotide antagonist encodes an antagonist of IL-10 and that such antagonist of IL-10 is a soluble or defective IL-10 receptor or IL-20/MDA-7, respectively. Claims 70-75 merely recite specific methods of directed evolution, such as synthetic ligation reassembly, gene site saturated mutagenesis, non-stochastic ligation reassembly, exonuclease-mediated reassembly, end selection, and shuffling respectively. Claims 76-80 and 83-84 merely recite that the immunomodulatory polynucleotide encodes an antigen, such as a cancer antigen, a bacterial antigen, a viral antigen, a parasite antigen, a self-antigen, or a cytokine or specific cytokines, respectively, all of which were known in the art. Claims 81-82 merely recite that the immune response is a humoral or cellular response, respectively, both of which were known in the art. As such, all of Claims 1, 2, 27-28 and 70-84 correspond to the Count.

**C. Claims which are dependent on Claims 1, 3 and 5**

Claims 7-26, 41-52, and 85-86 depend ultimately on any of Claims 1, 3, and 5, which are anticipated by the Count. Claims 7 and 8 merely recite that the polynucleotide is incorporated into a vector, or administered in conjunction with a vector, respectively, which were known in the art. Claim 9 merely states that the library is generated using gene reassembly or

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oligonucleotide-directed saturation mutagenesis, which were known in the art. Compared to Claim 5, which is anticipated by the Count, Claim 10 merely recites that the library is formed by reassembly of at least two parental templates, which was known in the art. Claim 11 merely recites conducting a further round of reassembly to produce a further library of recombinant polynucleotides and screening the further library, which method was also known in the art. Claims 12-16 merely recite that the polynucleotide encodes a polypeptide which interacts with a cellular receptor involved in mediating an immune response, such as a macrophage scavenger receptor, a cytokine or chemokine receptor, CCR5 or CCR6, or a polypeptide which mimics the activity of a natural ligand for the receptor but does not induce immune reactivity, respectively, which polypeptides were known in the art. Claims 17-19 merely recite that the library is displayed using a replicable genetic package, such as a phage, cell, spore or virus, such as M13 phage, respectively, which were known in the art. Claims 20 and 21 merely recite that the polynucleotide is inserted into, or introduced in conjunction with a genetic vaccine vector, respectively, which were known in the art. Claims 22 and 23 merely recite that the polynucleotide is inserted into an antigen-encoding sequence of a genetic vaccine vector, such as the M-loop of HBsAg, respectively, which was known in the art. Claim 24 merely recites that the polynucleotide encodes a sequence rich in unmethylated CpG, which was known in the art. Claims 25 and 26 merely recite that the polynucleotide encodes a polypeptide that inhibits an

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allergic reaction, such as an interferon, an interleukin or an antagonist thereof, respectively, which were known in the art.

Claims 41-45 merely recite that the polynucleotide encodes a cytokine antagonist, such as a soluble cytokine receptor or transmembrane cytokine receptor having a defective signal sequence, where the polynucleotide encodes a polypeptide which induces a T<sub>H</sub>1 or T<sub>H</sub>2 response, respectively, all of which were known in the art. Claims 46, 47 and 48 merely recite that the modulatory effect is a decreased unwanted effect, an increased desired effect, or both, respectively, which were known in the art. Claim 49 merely recites that the decreased unwanted effect and increased desired effect are evolved on a multimodule vaccine vector, which was known in the art. Claim 50 merely recites that the modulatory effect is increased stability of the immunomodulatory polynucleotide or encoded polypeptide, which was known in the art. Claims 51 and 52 merely recite that the optimized modulatory effect on an immune response is measured in a human or an animal host, respectively, which was known in the art. Claims 85-86 merely recite that the immune response is determined *in vitro* or *in vivo*, respectively, both of which were known in the art. As such, Claims 1, 3, 5, 7-26, 41-52 and 85-86 correspond to the Count.

**D. Claims which are dependent on Claims 1-6**

Claim 29 (which is anticipated by the Count), Claim 30 (which is one alternative of the Count), and Claims 31-40 ultimately depend from any of Claims 1-6. Claims 1-6 are anticipated

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by the Count. Claims 31-33 merely recite that the co-stimulator is CD1, CD40, CD154 or CD150, a cytokine, or specific cytokines, respectively, all of which were known in the art. Claims 34 and 35 merely recite that the library is screened by testing for activation of cells which contain a receptor for the cytokine, or a heterologous nucleic acid which encodes the receptor for the cytokine, respectively, which were known in the art. Claim 36 merely recites that the cytokine is IL-12, and the screening is by detecting T cell proliferation or differentiation, which were known in the art. Claim 37 merely recites that the cytokine is interferon- $\alpha$ , and the screening is by display using a replicable genetic package, which were known in the art. Claim 38 merely recites that immune response is differentiation of T cells to T<sub>H</sub>1 cells and the library is screened for members encoding a cytokine which induces cells to produce IL-2 and interferon- $\alpha$ , which were known in the art. Claim 39 merely recites that the cytokine encoded by the optimized polynucleotide exhibits reduced immunogenicity, and the reduced immunogenicity is detected by introducing a cytokine encoded by the polynucleotide into a mammal and determining whether an immune response is induced against the cytokine, which were known in the art. Claim 40 merely recites that the co-stimulator is B7-1 (CD80) or B7-2 (CD86), and the cell is tested for its ability to co-stimulate an immune response. B7-1 and B7-2 were known co-stimulators. As such, all of Claims 1-6 and 29-40 correspond to the Count.

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**E. Claims which are dependent on Claim 53**

Claims 54-58 all depend ultimately on Claim 53, which is obvious in view of the Count. Claim 54 merely recites that the screening involves introducing the library into a genetic vaccine vector that encodes an antigen and identifying cells exhibiting increased or decreased immunogenicity to the antigen, which steps were known in the art. Claims 55-58 merely recite that the accessory molecule comprises a proteasome or TAP polypeptide, a cytotoxic T-cell inducing sequence, specifically a sequence from HBsAg, or an immunogenic agonist sequence, respectively, which were known in the art. As such, all of Claims 53-58 correspond to the Count.

**F. Claims which are dependent on Claims 59-61**

Claims 62-66 all depend ultimately from any of Claims 59-61, which are obvious in view of the Count. Claims 62-66 merely recite that the recombinant expression host is a prokaryote, a eukaryote, a plant, a monocot or a dicot, respectively, which were known in the art. As such, all of Claims 59-66 all correspond to the Count.

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**G. Claims which are dependent on Claims 1, 3, 5, 53 and 59-61**

Claims 67-69 depend from any of Claims 1, 3, 5, 53 or 59-61, which are either anticipated by or obvious in view of the Count. Claims 67-69 merely recite that the library is created using gene site saturation mutagenesis, synthetic ligation polynucleotide reassembly or both, respectively, which were known in the art. As such, all of Claims 1, 3, 5, 53, 59-61 and 67-69 correspond to the Count.

**V. 35 U.S.C. § 135(b) IS SATISFIED**

At least one claim is being submitted in the above referenced application which is the same as, or for the same as, or substantially for the same subject matter as a claim of the '258 patent, and such claim (*e.g.*, Claim 47) was made prior to one year from the date on which the '258 patent was granted (*i.e.*, November 12, 2002) in an Amendment dated March 27, 2003. Thus, § 135(b) is satisfied.

**VI. CONCLUSION**

Applicants respectfully request that an interference be declared employing the proposed Count set forth on attached **Appendix B**, with Claims 1-86 of the '258 patent and Claim 47 of the present application designated as corresponding to the Count. Such action is respectfully requested.

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Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact Applicants' undersigned representative to expedite prosecution.

Respectfully submitted,

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**APPENDIX A**  
**EXEMPLARY SUPPORT FOR CLAIM 47<sup>3</sup>**

'869 Application Claim	Exemplary Support in the '869 Specification
<p>47. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:</p> <p>a) creating a library of recombinant polynucleotides; and</p> <p>b) screening the library to identify an optimized recombinant polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector;</p> <p>wherein the optimized recombinant polynucleotide or the polypeptide encoded by the recombinant polynucleotide exhibits an enhanced ability to modulate an immune response compared to a polynucleotide from which the library was created;</p>	<p>Claim 47 is one alternative of the Count. Support can be found in the '869 application at least in the title, at p. 3, l. 30 to p. 5, l. 5 and original claims 1 and step (2) of original claim 5.</p> <p>Support for step (a) can be found at least at p. 4, ll. 23-24 ("to produce a library of recombinant polynucleotides"); p. 11, ll. 3-11 and original claim 1.</p> <p>Support for step (b) can be found at least at p. 4, ll. 24-27 ("screening the library to identify at least one optimized recombinant polynucleotide....to modulate an immune response...."); p. 7, l. 24 to p. 8, l. 9; and original claim 1.</p> <p>Support for this clause can be located at least at p. 4, ll. 24-27 (see above) and claim 1 and step (2) of original claim 5.</p>

<sup>3</sup> The identified support is merely exemplary, and is not meant to be exhaustive. Applicants reserve the right to recite additional support for these claims at a later time, if necessary.

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'869 Application Claim	Exemplary Support in the '869 Specification
<p>wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector,</p> <p>wherein the optimized recombinant polynucleotide encodes a co-stimulator selected from B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4,</p> <p>and whereby optimization is achieved by recursive sequence recombination.</p>	<p>Support for this clause can be located at least in original claim 1 ("... to identify an optimized recombinant polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vaccine genetic vector;").</p> <p>Support for this clause can be located at least at p. 4, ll. 6-8 (examples of polynucleotides encoding co-stimulators, including, e.g., B7-1 and B7-2); p. 5, l. 31 to p. 6, l. 8; p. 16, ll. 24-28; p. 39, ll. 14-19; p. 46, ll. 23-25; p. 49, l. 13 to p. 53, l. 20; Example 1; Figures 10, 11 and 15; and original claims 24 ("the optimized recombinant polynucleotide encodes a costimulatory") and 25 ("screening step involves selecting variants with altered activity through CD28 or CTLA-4").</p> <p>Support can be located at least at p. 17, ll. 21-31 ("recursive sequence recombination can be employed to achieve still further improvements in a desired property" and "Recursive sequence recombination entails successive cycles of recombination to generate molecular diversity."); p. 18, ll. 18-30.</p>

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## APPENDIX B

<u>SOURCE</u>	<u>PROPOSED COUNT</u>
<b>Claim 30 of the '258 patent</b>	30. The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4. <sup>4</sup>

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<sup>4</sup> Claim 30 depends directly from Claim 29, which depends in turn from any of Claims 1-6. The limitations of Claims 5,6 and 29 have been included in Claim 47, the second alternative of the Proposed Count. Claims 5, 6 and 29 are recited below:

**5.** A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:

a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and

b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; and

whereby optimization is achieved using one or more directed evolution methods in any combination, permutation, and iterative manner.

**6.** The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

**29.** The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

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SOURCE

PROPOSED COUNT

OR

**Claim 47 of the  
'869 application**

47. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:

a) creating a library of recombinant polynucleotides; and

b) screening the library to identify an optimized recombinant polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector;

wherein the optimized recombinant polynucleotide or the polypeptide encoded by the recombinant polynucleotide exhibits an enhanced ability to modulate an immune response compared to a polynucleotide from which the library was created;

wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector, wherein the optimized recombinant polynucleotide encodes a co-stimulator selected from B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4, and whereby optimization is achieved by recursive sequence recombination.

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<sup>4</sup>(...continued)  
generated polynucleotide encodes a co-stimulator.

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**APPENDIX C**  
**COMPARISON OF THE '258 PATENT CLAIMS WITH THE PROPOSED COUNT**

The following assumption is being made with respect to the '258 claims:

The state of the art is based on the filing date of the Short '052 application, which issued as the '258 patent, *i.e.* January 31, 2000.<sup>5</sup>

'258 Patent Claims	Claim Correspondence to the Proposed Count
<p>1. A method for obtaining an immuno-modulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:  creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, wherein optimization is achieved by at least one directed evolution method in any combination, permutation and iterative manner.</p>	<p>Claim 30 of the '258 patent, which is part of the Count, depends from Claim 29, which depends from any of Claims 1-6. Claim 30, which includes all of the recitations of Claim 1, is a species of Claim 1. Furthermore, the recitations of Claim 1 are included in their entireties in Claim 47 of the '869 application, which is likewise part of the Count. Accordingly, Claim 1 is anticipated by the Count.</p>

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<sup>5</sup> As previously noted, the '258 patent was substantially copied from at least four PCT publications of the assignee of the present '869 application ("Maxygen"), including PCT/US99/03020 (WO 99/41368), which corresponds to the disclosure of the present '869 application, PCT/US99/03023 (WO 99/41402), PCT/US99/02944 (WO 99/41383), and PCT/US99/03022 (WO 99/41369), all of which were published August 19, 1999. Accordingly, the Short '052 application can only be accorded the benefit of its filing date, January 31, 2000 as indicated in Appendix F.

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'258 Patent Claims	Claim Correspondence to the Proposed Count
<p>2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.</p>	<p>Claim 30 of the '258 patent, which is part of the Count, depends from Claim 29, which depends from any of Claims 1-6. Claim 30, which includes all of the recitations of Claim 2, is a species of Claim 2. Furthermore, the recitations of Claim 2 are included in their entireties in Claim 47 of the '869 application, which is likewise part of the Count. Accordingly, Claim 2 is anticipated by the Count.</p>
<p>3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:</p> <p style="margin-left: 40px;">screening a library Qf [sic] non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.</p>	<p>Claim 30 of the '258 patent, which is part of the Count, is dependent on Claim 29, which is dependent on any of Claims 1-6. Claim 30, which includes all of the limitations of Claim 3, is a species of Claim 3. Furthermore, the recitations of Claim 3 are included in their entireties in Claim 47 of the '869 application, which is likewise part of the Count. Thus, Claim 3 is anticipated by the Count.</p>

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'258 Patent Claims	Claim Correspondence to the Proposed Count
<p><b>4.</b> The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.</p>	<p>Claim 30 of the '258 patent, which is part of the Count, depends from Claim 29, which depends from any of Claims 1-6. Claim 30, which includes all of the limitations of Claim 4, therefore is a species of Claim 4. Additionally, the limitations of Claim 4 are recited in Claim 47 of the '869 application, which is part of the Count. Thus, Claim 4 is anticipated by the Count.</p>
<p><b>5.</b> A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:</p> <p>a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and</p> <p>b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was</p>	<p>Claim 30 of the '258 patent, which is part of the Count, is dependent ultimately from Claim 5, and thus necessarily contains all the recitations of Claim 5. Moreover, the recitations of Claim 5 are included in their entireties in Claim 47 of the '869 application, which is likewise part of the Count. Thus, Claim 5 is anticipated by the Count.</p>

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<p>created; and</p> <p>whereby optimization is achieved using one or more directed evolution methods in any combination, permutation, and iterative manner.</p>	
<p>6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.</p>	<p>Claim 30 of the '258 patent, which is part of the Count, is dependent on Claim 29, which is dependent on any of Claims 1-6, and thus necessarily contains all the recitations of Claim 6. Moreover, the recitations of Claim 6 are included in their entireties in Claim 47 of the '869 application, which is likewise part of the Count. Thus, Claim 6 is anticipated by the Count.</p>
<p>7. The method of any claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide is incorporated into a vector.</p>	<p>Claim 7 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count and thus corresponds to the Count. Claim 7 merely adds that the optimized polynucleotide is incorporated into a vector. Techniques for cloning polynucleotide sequences into vectors and isolating vectors were well known. <i>See, e.g.,</i> Sambrook <i>et al.</i>, MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory Press, New York (2d ed. 1989), pp. 1.53-1.59. Specifying that the polynucleotide is incorporated into a vector does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 7 is obvious over the Count.</p>
<p>8. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide</p>	<p>Claim 8 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count as discussed above. Claim 8 simply adds that the</p>

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encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a vector.	optimized polynucleotide, or a polypeptide encoded by the optimized polynucleotide, is administered in conjunction with a vector. Methods involving administering a polynucleotide encoding a polypeptide with a vector were well known. <i>See, e.g., Kim et al., "In Vivo Engineering of a Cellular Response by Coadministration of IL-12 Expression Vector with a DNA immunogen," J. Immunol.</i> 158(2):816-26 (1997). Methods involving administering a polypeptide with a vector were also well known. <i>See, e.g., Donnelly et al., "DNA Vaccines," Annu. Rev. Immunol.</i> 15:617-48, 620 (1997). Merely providing that the optimized polynucleotide or polypeptide encoded by the optimized polynucleotide is administered with a vector does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 8 is obvious over the Count.
9. The method of any of claims 1, 3 or 5, wherein the library of non-stochastically generated progeny polynucleotides is created by a process selected from gene reassembly or oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner.	Claim 9 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 9 merely adds that the library of progeny polynucleotides is created by a process selected from gene reassembly or oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner. It was well known in the art that progeny polynucleotides could be created from a set of parental polynucleotides by oligonucleotide-directed saturation mutagenesis. <i>See, e.g., Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL (1989), supra, pp. 15.51-15.113 (see especially 15.95-15.108); Hill et al., "Mutagenesis with Degenerate</i>

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	<p>Oligonucleotides: An Efficient Method for Saturating a Defined DNA Region with Base Pair Substitutions," <i>in METHODS IN ENZYMOLOGY: RECOMBINANT DNA</i> 155: 558-568 (Ray Wu ed., Acad. Press, Inc., 1987); Goff <i>et al.</i>, "Laboratory Methods: Efficient Saturation Mutagenesis of a Pentapeptide Coding Sequence Using Mixed Oligonucleotides," <i>DNA</i> 6(4):381-388 (1987); and Murray <i>et al.</i>, "Saturation mutagenesis of a major histocompatibility complex protein domain: Identification of a single conserved amino acid important for allorecognition," <i>PNAS USA</i> 85:3535-39 (1988); WO 99/41368; and Horwitz <i>et al.</i>, <i>in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY</i> 185: 599-611 (David V. Goeddel ed., Acad. Press, Inc. 1990). Gene reassembly methods were also well known and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i>, WO 98/27230 and WO 99/41368. Thus, the additional limitation that the library of progeny polynucleotides is created by gene reassembly or oligonucleotide-directed saturation mutagenesis does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 9 is obvious over the Count.</p>
<p>10. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by:</p> <p>a) non-stochastically reassembling at</p>	<p>Claim 10 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 30, which is part of the Count, depends ultimately from Claims 1-6, and thus necessarily contains all of the limitations of Claim 5. Claim 5 specifies in step (a) creating a library of non-stochastically generated</p>

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<p>least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response;</p> <p>wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and</p> <p>b) screening the library to identify at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.</p>	<p>progeny polynucleotides from a parental set. Compared to Claim 5, Claim 10 merely adds that the library of non-stochastically generated polynucleotides is generated by non-stochastically reassembling at least two parental polynucleotides that differ from each other in two or more nucleotides. Methods of non-stochastically reassembling at least two parental polynucleotides that differ from each other in two or more nucleotides were well known and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i>, WO 98/27230 and WO 99/41368. This additional limitation does not provide a patentable distinction over at least Claim 5, which is anticipated by the Count. Thus, Claim 10 is obvious over the Count.</p>
<p>11. The method of claim 10, wherein the method further comprises the steps of:</p> <p>c) subjecting an optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further library of recombinant polynucleotides;</p> <p>d) screening the library produced in c) to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an</p>	<p>Claim 11 depends from Claim 10, which is obvious over the Count. Claim 10, as noted above, depends from Claim 1, 3, or 5, each of which is anticipated by the Count. Compared to Claim 10, Claim 11 simply provides conducting a further round of reassembly step to produce a further library of recombinant polynucleotides and screening this further library to identify at least one further optimized polynucleotide that exhibits an enhanced ability to modulate an immune response. Merely repeating these steps does not provide a patentable distinction over Claim 10, from which Claim 11 depends. <i>See, e.g.</i>, WO 98/27230 and WO 99/41368. As Claim 11 is obvious over Claim 10, Claim 11 also is</p>

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immune response in comparison to a parental polynucleotide from which the library was created; and  e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.	obvious over the Count.
12. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.	Claim 12 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 12 merely adds that the optimized polynucleotide encodes a polypeptide that interacts with a cellular receptor involved in mediating an immune response, and that the polypeptide acts as an agonist or antagonist of the receptor. Polypeptides that interact with cellular receptors involved in mediating immune responses and act as agonists and antagonists of such cellular receptors were well known. See, e.g., Paul <i>et al.</i> , "Lymphocyte responses and cytokines," <i>Cell</i> 76: 241-251, 241-242 (1994); Baggolini <i>et al.</i> , <i>Annu. Rev. Immunol.</i> 15: 675-705, 675 (1997); Greenfeder <i>et al.</i> , "Insertion of a Structural Domain of Interleukin (IL)-1 $\alpha$ Confers Agonist Activity to the IL-1 Receptor Antagonist," <i>J. Biol. Chem.</i> 270(38): 22460-6 (1995); Thomas <i>et al.</i> , "Potent interleukin 3 receptor agonist with selectively enhanced hematopoietic activity relative to recombinant human interleukin 3," <i>PNAS USA</i> 92: 3779-83 (1995); Livnah <i>et al.</i> , "Functional Mimicry of a Protein Hormone by

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	<p>a Peptide Agonist: The EPO Receptor Complex at 2.8 ,” <i>Science</i> 273: 464-71 (1996); and Cwirla <i>et al.</i>, “Peptide Agonist of the Thrombopoietin Receptor as Potent as the Natural Cytokine,” <i>Science</i> 276: 1696-9 (1997). Merely adding that the polynucleotide encodes a polypeptide that interacts with a cellular receptor and acts as an agonist or antagonist of the receptor does not provide a patentable distinction over Claim 1, 3 or 5, each of which is anticipated by the Count. Thus, Claim 12 is obvious over the Count.</p>
<p>13. The method of claim 12, wherein the cellular receptor is a macrophage scavenger receptor.</p>	<p>Claim 13 depends from Claim 12, which is obvious over the Count. Claim 13 merely adds that the cellular receptor is a macrophage scavenger receptor. Macrophage scavenger receptors were well known cellular receptors having broad binding specificity for a variety of ligands. <i>See, e.g.</i>, Krieger <i>et al.</i>, “Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP),” <i>Annu. Rev. Biochem.</i> 63: 601-37 (1994). Accordingly, Claim 13 does not patentably distinguish over Claim 12, which is obvious over the Count. Thus, Claim 13 is obvious over the Count.</p>
<p>14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.</p>	<p>Claim 14 depends from Claim 12, which is obvious over the Count. Claim 14 merely specifies that the cellular receptor is a cytokine receptor or a chemokine receptor. Cytokine receptors were well known cellular receptors. <i>See, e.g.</i>, HUMAN CYTOKINES: HANDBOOK FOR BASIC AND CLINICAL RESEARCH, Vol. II (Aggarwal &amp; Guterman eds. 1996); and Paul <i>et</i></p>

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	<i>al.</i> , <i>Cell</i> 76: 241-251, 241-242 (1994). Chemokine receptors were also well known cellular receptors. See, e.g., Baggolini <i>et al.</i> , <i>Annu. Rev. Immunol.</i> 15: 675-705, 675 (1997); Horuk, <i>TIPS</i> 15: 159-165, 159 (1994); Paul <i>et al.</i> , <i>Cell</i> 76: 241-251, 241-242 (1994); and Premack <i>et al.</i> , <i>Nature Med.</i> 2(11): 1174-1178, 1174 (1996). As Claim 14 does not patentably distinguish over Claim 12, Claim 14 is obvious over the Count.
15. The method of claim 14, wherein the chemokine receptor is CCR5 or CCR6.	Claim 15 depends from Claim 14, which is obvious over the Count. The only limitation added by Claim 15 is that the chemokine receptor is CCR5 or CCR6. CCR5 and CCR6 were well known chemokine receptors. See, e.g., Baggolini <i>et al.</i> , <i>Annu. Rev. Immunol.</i> 15:675-705, 676 (1997); Yoshie <i>et al.</i> , <i>J. Leukocyte Biol.</i> 62:634-644, 634 (1997); Cohen <i>et al.</i> , "Host factors in the pathogenesis of HIV disease," <i>Immunol. Rev.</i> 159:31-48 (1997); and Simmons <i>et al.</i> , "Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist," <i>Science</i> 276 (5310):276-9 (1997). Therefore, Claim 15 does not add a patentable distinction over Claim 14 given the state of the art and is obvious over the Count.
16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.	Claim 16 depends from Claim 12, which is obvious over the Count. The only limitation added by Claim 16 is that the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to that natural ligand. It was known that one could make small polypeptides that are

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	structurally unrelated to a natural ligand for a cellular receptor and thus do not induce immune reactivity to the natural ligand, but that functionally mimic the activity of the natural ligand for the receptor. <i>See, e.g.,</i> the Livnah <i>et al.</i> and Cwirla <i>et al.</i> references cited for Claim 12. Because this limitation does not patentably distinguish over Claim 12, which is obvious over the Count, Claim 16 is also obvious over the Count.
17. The method of claim 12, wherein the library is screened by: <ul style="list-style-type: none"><li data-bbox="204 994 758 1184">i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package;</li><li data-bbox="204 1216 709 1332">ii) contacting the replicable genetic packages with a plurality of cells that display the receptor; and</li><li data-bbox="204 1364 677 1469">iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.</li></ul>	Claim 17 depends from Claim 12, which is obvious over the Count. Claim 17 simply adds that the library is screened by expressing polynucleotides of the library so that the encoded polypeptides are produced as fusions with a protein that is displayed on the surface of a replicable genetic package, contacting the replicable genetic packages with cells that display the receptor, and identifying cells that exhibit modulation of an immune response mediated by the receptor. Techniques for screening a library of biologically active proteins by expressing polypeptides as fusions with a protein displayed on the surface of a replicable genetic package, such as a bacteriophage, spore, or virus, contacting such displayed polypeptides with cellular receptors, and identifying those displayed polypeptides that bind the receptors and exhibit biological activity were well known. <i>See, e.g.,</i> Cwirla <i>et al.</i> , "Peptides on phage: A vast library of peptide for identifying ligands," <i>PNAS USA</i> 87:6378-6382 (1990); Kay <i>et al.</i> , <i>PHAGE DISPLAY OF PEPTIDES AND PROTEINS: A LABORATORY MANUAL</i> 24-26 (Academic Press)

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	1996); U.S. Pat. No. 5,571,698; Han <i>et al.</i> , PNAS USA 92:9747-9751 (1995); WO 94/18330; and WO 94/01567. Thus, the steps added by Claim 17 do not patentably distinguish over Claim 12, which is obvious over the Count. Thus, Claim 17 is obvious over the Count.
18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.	Claim 18 depends from Claim 17, which is obvious over the Count. Claim 18 merely adds that the replicable genetic package is a bacteriophage, cell, spore, or virus. Spores, cells, and bacteriophage were well known replicable genetic packages. <i>See, e.g.</i> , Kay <i>et al.</i> , PHAGE DISPLAY OF PEPTIDES AND PROTEINS: A LABORATORY MANUAL (Academic Press 1996); U.S. Pat. No. 5,571,698, Col. 7, lines 37-38 and Col. 47, line 65 to Col. 48, line 22; U.S. Pat. No. 5,348,867. Thus, the limitation added by Claim 18 does not patentably distinguish over Claim 17, and Claim 18 is obvious over the Count.
19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.	Claim 19 depends from Claim 18, which is obvious over the Count. The only limitations added by Claim 19 are that the replicable genetic package is an M13 bacteriophage, and the protein is encoded by gene III or gene VIII. These limitations do not patentably distinguish over Claim 18, because M13 bacteriophage and its coat proteins ( <i>e.g.</i> , the M13 gene III or gene VIII proteins) were well known. Methods involving inserting libraries encoding polypeptides to be displayed into either gene III or gene VIII of M13 phage to form fusion proteins were also well known. <i>See, e.g.</i> , U.S.

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	Pat. No. 5,571,698, Col. 48, lines 45-65; WO 91/19818; WO 92/01047 (gene III); WO 92/06204; WO 92/18619 (gene VIII); McLafferty <i>et al.</i> , "M13 bacteriophage displaying disulfide-constrained microproteins," <i>Gene</i> 128(1): 29-36 (1993); Cortese <i>et al.</i> , "Selection of biologically active peptides by phage display of random peptide libraries," <i>Curr. Opin. Biotechnol.</i> 7(6): 616-21 (1996); and Dunn, "Phage display of proteins," <i>Curr. Opin. Biotechnol.</i> 7(5): 547-53 (1996). As Claim 19 does not patentably distinguish over Claim 18, which is obvious over the Count, Claim 19 also is obvious over the Count.
20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.	Claim 20 depends from Claim 12, which is obvious over the Count. Claim 20 simply adds that the optimized polynucleotide is introduced into a genetic vaccine vector, and the vector is administered to a mammal. Methods for introducing a polynucleotide sequence encoding a polypeptide into a genetic vaccine vector ( <i>e.g.</i> , DNA vaccine vector) and administering the resultant vector to a mammal for expression of the polypeptide were well known. <i>See, e.g.</i> , Chow <i>et al.</i> , "Improvement of Hepatitis B Virus DNA Vaccines by Plasmids Coexpressing Hepatitis B Surface Antigen and Interleukin-2," <i>J. Virol.</i> 71(1): 169-78 (1997); and Donnelly <i>et al.</i> , "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-48, 620 (1997). As Claim 20 does not patentably distinguish over Claim 12, which is obvious over the Count, Claim 20 also is obvious over the Count.

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<p><b>21.</b> The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.</p>	<p>Claim 21 depends from Claim 12, which is obvious over the Count. Claim 21 merely adds that the optimized polynucleotide is introduced into a mammal in conjunction with a genetic vaccine vector. Methods comprising co-administration of a polypeptide with a genetic vaccine vector, such as a DNA vaccine vector, were known and would have been obvious to the skilled artisan. <i>See, e.g.,</i> Donnelly <i>et al.</i>, "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-648, 620 (1997). As Claim 21 does not patentably distinguish over Claim 12, which is obvious over the Count, Claim 21 also is obvious over the Count.</p>
<p><b>22.</b> The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.</p>	<p>Claim 22 depends from Claim 12, which is obvious over the Count. Claim 22 merely provides that the optimized polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector. Methods for introducing a polypeptide-encoding polynucleotide into a nucleotide sequence of a genetic vaccine vector were well known. <i>See, e.g.,</i> Donnelly <i>et al.</i>, "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-48, 620 (1997). Furthermore, insertion of a foreign sequence into an antigen-encoding nucleotide sequence was a common practice. <i>See, e.g.,</i> Pumpens <i>et al.</i>, "Hepatitis B virus core particles as epitope carriers," <i>Intervirology</i> 38(1-2): 63-74 (1995); and Ulrich <i>et al.</i>, "Chimeric HBV core particles carrying a defined segment of Puumala hantavirus nucleocapsid protein evoke protective immunity in an animal model," <i>Vaccine</i> 16(2-3): 272-80 (1998). As Claim 22 does not patentably distinguish over Claim 12,</p>

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	which is obvious over the Count, Claim 22 also is obvious over the Count.
23. The method of claim 22, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an M-loop of an HBsAg polypeptide.	Claim 23 depends from Claim 22, which is obvious over the Count. Claim 23 simply specifies that the optimized polynucleotide is introduced into a nucleotide sequence that encodes an M-loop of an HBsAg polypeptide. As discussed for Claim 22, methods for introducing a foreign sequence into the nucleotide sequence of a genetic vaccine vector, including an antigen-encoding nucleotide sequence of a genetic vaccine vector, would have been obvious to the skilled artisan. The antigenic M loop of hepatitis B surface antigen, which is recognized by the monoclonal antibody RFHB7 would have been an obvious choice to use in this fashion to a skilled artisan. <i>See, e.g., Chen et al., "Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library," PNAS USA 93(5): 1997-2001 (1996).</i> Claim 23 does not provide a patentable distinction over Claim 22, which is obvious over the Count. Accordingly, Claim 23 is obvious over the Count.
24. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.	Claim 24 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 24 merely adds that the optimized polynucleotide comprises a nucleotide sequence rich in unmethylated CpG. Immunostimulatory oligodeoxynucleotides containing the unmethylated CpG motif were well known. <i>Weiner et al., "Immunostimulatory oligodeoxynucleotides containing the CpG motif are effective as immune adjuvants in</i>

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	<p>tumor antigen immunization," <i>PNAS USA</i> 94: 10833-7 (1997); and Pisetsky, "Immune Activation by Bacterial DNA: A New Genetic Code," <i>Immunity</i> 5: 303-10 (1996); Klinman <i>et al.</i>, "Contribution of CpG Motifs to the Immunogenicity of DNA Vaccines," <i>J. Immunol.</i> 158(8): 3635-39 (1997). This limitation does not patentably distinguish over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 24 is obvious over the Count.</p>
25. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that inhibits an allergic reaction.	<p>Claim 25 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 25 simply adds that the optimized polynucleotide encodes a polypeptide that inhibits an allergic reaction. A variety of polypeptides able to inhibit an allergic response were known. For example, certain cytokines were known to inhibit allergic reactions, such as interferon-<math>\alpha</math>, interferon-<math>\gamma</math>, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13, all of which are recited in Claim 26. <i>See, e.g.</i>, Brusselle <i>et al.</i>, "Role of IFN-<math>\gamma</math> in the Inhibition of Allergic Airway Inflammation Caused by IL-12," <i>Am. J. Respir. Cell Mol. Biol.</i> 17: 767-71 (1997). Thus, such polypeptides would have been obvious targets to one skilled in the art for purposes of obtaining optimized polynucleotides encoding polypeptides that inhibit allergic reactions. Accordingly, Claim 25 does not provide a patentable distinction over any of Claims 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 25 is obvious over the Count.</p>

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<p><b>26.</b> The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon-<math>\alpha</math>, interferon-<math>\gamma</math>, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.</p>	<p>Claim 26 depends from Claim 25, which is obvious over the Count. Claim 26 merely adds that the polypeptide that inhibits an allergic response is interferon-<math>\alpha</math>, interferon-<math>\gamma</math>, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, or an antagonist of IL-13. It was well known that each of these polypeptides inhibited an allergic reaction. <i>See, e.g.,</i> Brusselle <i>et al.</i>, "Role of IFN-<math>\gamma</math> in the Inhibition of Allergic Airway Inflammation Caused by IL-12," <i>Am. J. Respir. Cell Mol. Biol.</i> 17: 767-71 (1997); Gauchat <i>et al.</i>, "Regulation of human IgE synthesis: the role of CD4+ and CD8+ T-cells and the inhibitory effects of interferon-alpha," <i>Eur. Respir. J. Suppl.</i> 13: 31s-38s (1991); Parronchi <i>et al.</i>, "IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones," <i>J. Immunol.</i> 149(9): 2977-83 (1992); Grunig <i>et al.</i>, "Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis," <i>J. Exp. Med.</i> 185(6): 1089-99 (1997); Stern <i>et al.</i>, Chap. 4, <i>Interleukin-12, in HUMAN CYTOKINES</i>. HANDBOOK FOR BASIC AND CLINICAL RESEARCH 74-96 (Aggarwal &amp; Guterman eds., 1996); Devos <i>et al.</i>, "Interleukin-5 and its receptor: a drug target for eosinophilia associated with chronic allergic disease," <i>J. Leukoc. Biol.</i> 57(6): 813-19 (1995); de Vries <i>et al.</i>, "Novel fundamental approaches to intervening in IgE-mediated allergic diseases," <i>J. Invest. Dermatol.</i> 102(2): 141-4 (1994); de Vries <i>et al.</i>, <i>Interleukin-4 and Interleukin-13</i>,</p>

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	<p>Chap. 8, <i>in CYTOKINE REGULATION OF HUMORAL IMMUNITY: BASIC AND CLINICAL ASPECTS</i> 195-215 (C. M. Snapper, West Sussex, UK, John Wiley and Sons, 1996); and de Vries <i>et al.</i>, "Modulation of the human IgE response," <i>Eur. Respir. J. Suppl.</i> 22: 58s-62s (1996). Thus, these polypeptides would have been obvious targets to one skilled in the art for purposes of obtaining optimized polynucleotides encoding polypeptides that inhibit allergic reactions. Claim 26 does not patentably distinguish over Claim 25, which is obvious over the Count. Accordingly, Claim 26 is obvious over the Count.</p>
<p><b>27.</b> The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.</p>	<p>Claim 27 depends from Claim 1, which is anticipated by the Count. Claim 27 merely adds that the optimized recombinant polynucleotide encodes an antagonist of IL-10. IL-10 antagonists were well known and thus would have been obvious targets to the skilled artisan for purposes of obtaining an optimized recombinant polynucleotide encoding an IL-10 antagonist. <i>See, e.g.</i>, Tan <i>et al.</i>, "Characterization of IL-10 Receptors on Human and Mouse Cells," <i>J. Biol. Chem.</i> 268(28): 21053-59 (1993); and Tan <i>et al.</i>, "Characterization of recombinant extracellular domain of human interleukin-10 receptor." <i>J. Biol. Chem.</i> 270(21): 12906-11 (1995). Claim 27 does not patentably distinguish over Claim 1, which is anticipated by the Count. Thus, Claim 27 is obvious over the Count.</p>
<p><b>28.</b> The method of claim 27, wherein the antagonist of IL-10 is soluble or defective</p>	<p>Claim 28 depends from Claim 27, which is obvious over the Count. Claim 28 merely adds</p>

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IL-10 receptor or IL-20/MDA-7.	that the IL-10 antagonist is soluble or defective IL-10 receptor or IL-20/MDA-7. Soluble antagonists of IL-10 ( <i>e.g.</i> , soluble IL-10 receptors) were well known antagonists of IL-10 and would have been an obvious choice to one skilled in the art. <i>See, e.g.</i> , Tan <i>et al.</i> , “Characterization of recombinant extracellular domain of human interleukin-10 receptor,” <i>J. Biol. Chem.</i> 270(21): 12906-11 (1995). IL-20/MDA-7 was also well known and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i> , Jiang <i>et al.</i> , “Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression,” <i>Oncogene</i> 11(12): 2477-86 (1995); and De Waal Malefyt <i>et al.</i> , “A Novel Cytokine Belonging to the IL-10 Gene Family Affects Human Monocytes and T Cells,” Abstract, 13th European Immunology Meeting, Amsterdam, Netherlands, June 1997, <i>Immunol. Letters</i> 56(1): 211 (May 1997). As Claim 28 does not provide a patentable distinction over Claim 27, which is obvious over the Count, Claim 28 also is obvious over the Count.
29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.	Claim 30, which is an alternative of the Count, depends from Claim 29, and thus necessarily includes all of the limitations of Claim 29. Moreover, the recitations of Claim 29 are included in their entireties in Claim 47 of the ‘869 application, which is likewise part of the Count. Thus, Claim 29 is anticipated by the Count.

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<b>30.</b> The method of claim 29, wherein the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4.	Claim 30 is one alternative of the Count.
<b>31.</b> The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).	<p>Claim 31 depends from Claim 29, which is anticipated by the Count. Claim 31 simply adds that the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM). Each of these co-stimulators was known in the art and thus would have been an obvious choice to one skilled in the art. <i>See, e.g.,</i> Porcelli, "The CD1 family: a third lineage of antigen-presenting molecules," <i>Adv. Immunol.</i> 59: 1-98 (1995); Foy <i>et al.</i>, "Immune regulation by CD40 and its ligand GP39," <i>Annu. Rev. Immunol.</i> 14: 591-617 (1996); Grewal <i>et al.</i>, "The CD40-CD154 system in anti-infective host defense," <i>Curr. Opin. Immunol.</i> 9(4): 491-7 (1997); and Aversa <i>et al.</i>, "SLAM and its role in T cell activation and Th cell responses." <i>Immunol. Cell Biol.</i> 75(2): 202-5 (1997). Thus, Claim 31 does not provide a patentable distinction over Claim 29 and is obvious over the Count.</p>
<b>32.</b> The method of claim 29, wherein the co-stimulator is a cytokine.	<p>Claim 32 depends from Claim 29, which is anticipated by the Count. Claim 32 only adds that the co-stimulator is a cytokine. Cytokines were well known as important regulators and co-stimulators of the immune system. <i>See, e.g.,</i> Kroemer <i>et al.</i>, "Immunoregulation by cytokines," <i>Crit. Rev. Immunol.</i> 13(2): 163-91 (1993). As Claim 32 does not provide a patentable distinction over Claim 29, which is</p>

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	anticipated by the Count, Claim 32 also is obvious over the Count.
33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , and IL-20 (MDA-7).	Claim 33 depends from Claim 32, which is obvious over the Count. Claim 33 merely specifies the cytokine to be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , or IL-20 (MDA-7). Each of these cytokines was well known and would have been an obvious choice to one skilled in the art. <i>See, e.g., HUMAN CYTOKINES: HANDBOOK FOR BASIC AND CLINICAL RESEARCH</i> , Vols. I-II (Aggarwal & Guterman eds. 1996), which describes IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, GM-CSF, G-CSF, TNF- $\alpha$ , IFN- $\alpha$ , and IFN- $\gamma$ . IL-16, IL-17, IL-18, and IL-20/MDA-7 were also known and would have been an obvious choice to a skilled artisan. <i>See, e.g., Laberge et al., J. Immunol.</i> 156(1): 310-5 (1996); <i>Yao et al., "Human IL-17: A Novel Cytokine Derived from T Cells," J. Immunol.</i> 155(12): 5483-6 1995; <i>Udagawa et al., "Interleukin-18 (interferon-gamma-inducing factor) is produced by osteoblasts and acts via granulocyte/macrophage colony-stimulating factor and not via interferon-gamma to inhibit osteoclast formation," J. Exp. Med.</i> 185(6): 1005-12 (1997); <i>Jiang et al., "Subtraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression," Oncogene</i> 11(12): 2477-86 (1995); and De

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	Waal Malefyt <i>et al.</i> , "A Novel Cytokine Belonging to the IL-10 Gene Family Affects Human Monocytes and T Cells," Abstract, 13th European Immunology Meeting, Amsterdam, Netherlands, June 1997, <i>Immunol. Letters</i> 56(1): 211 (1997). Claim 33 does not provide a patentable distinction over Claim 32, which is obvious over the Count. Accordingly, Claim 33 is obvious over the Count.
34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.	Claim 34 depends from Claim 33, which is obvious over the Count. Claim 34 merely adds that the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the polynucleotides to activate cells that contain a receptor for the cytokine. It was well known that cytokines signal through cellular receptors. <i>See, e.g., Kroemer et al., "Immunoregulation by cytokines," Crit. Rev. Immunol.</i> 13(2): 163-91 (1993); and Ihle <i>et al.</i> , "Signaling through the hematopoietic cytokine receptors," <i>Annu. Rev. Immunol.</i> 13: 369-98 (1995). As Claim 34 does not provide a patentable distinction over Claim 33, which is obvious over the Count, Claim 34 also is obvious over the Count.
35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.	Claim 35 depends from Claim 34, which is obvious over the Count. Claim 35 merely adds that the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine. It was well known by those skilled in the pertinent art that nucleotide sequences encoding cytokine receptors could be readily be cloned and expressed in cells and such cellular receptors could be used as targets for the

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	<p>identification of new cytokines. <i>See, e.g.</i>, Herz <i>et al.</i>, "Molecular approaches to receptors as targets for drug discovery," <i>J. Recept. Signal Transduct. Res.</i> 17(5): 671-776 (1997). Furthermore, it would have been obvious to one skilled in the art that cells expressing a cytokine receptor could be made by transfecting such cells with a nucleic acid encoding the cytokine. Thus, Claim 35 does not patentably distinguish over Claim 34, which is obvious over the Count. Accordingly, Claim 35 is obvious over the Count.</p>
<p><b>36.</b> The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.</p>	<p>Claim 36 depends from Claim 33, which is obvious over the Count. Claim 36 adds that the cytokine is IL-12 and the screening is performed by growing mammalian cells containing the genetic vaccine vector in a culture medium and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium. It was known that the activity of recombinant IL-12 could be demonstrated <i>in vitro</i> through the ability of the culture medium to induce proliferation of T cell clones (<i>see, e.g.</i>, Bramson <i>et al.</i>, "Construction of a double recombinant adenovirus vector expressing a heterodimeric cytokine: <i>in vitro</i> and <i>in vivo</i> production of biologically active interleukin-12," <i>Hum. Gene Ther.</i> 7(3): 333-42 (1996)). It was also known that delivery of IL-12 with a DNA vaccine vector induced a T cell proliferation response. Kim <i>et al.</i>, "In Vivo Engineering of a Cellular Response by Coadministration of IL-12 Expression Vector with a DNA immunogen," <i>J. Immunol.</i> 158(2):</p>

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	816-26 (1997). Thus, Claim 36 does not provide a patentable distinction over Claim 33, which is obvious over the Count. Accordingly, Claim 36 is obvious over the Count.
37. The method of 33, wherein the cytokine is interferon- $\alpha$ and the screening is performed by:  i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package;  ii) contacting the replicable genetic packages with a plurality of B cells; and  iii) identifying phage library members that are capable of inhibiting proliferation of the B cells.	Claim 37 depends from Claim 33, which is obvious over the Count. Claim 37 merely specifies that the cytokine is IFN- $\alpha$ , and the screening is performed by expressing the polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package, contacting the replicable genetic packages with a plurality of B cells, and identifying phage library members capable of inhibiting proliferation of the B cells. It was also known that IFN- $\alpha$ inhibits proliferation of B cells. See, e.g., Basham <i>et al.</i> , "Synergistic antitumor activity with IFN and monoclonal anti-idiotype for murine B cell lymphoma. Mechanism of action," <i>J. Immunol.</i> 141(8): 2855-60 (1988); and Randhawa <i>et al.</i> , "In vitro culture of B-lymphocytes derived from Epstein-Barr-virus-associated posttransplant lymphoproliferative disease: cytokine production and effect of interferon-alpha," <i>In Vitro Cell Dev. Biol. Anim.</i> 33(10): 803-8 (1997). It would have been obvious to one skilled in the pertinent art that the encoded polypeptides could be produced as fusions with a protein displayed on the surface of a replicable genetic package and screened for inhibition of proliferation of B cells. See, e.g., Cwirla <i>et al.</i> , <i>PNAS USA</i> 87: 6378-82 (1990); and Kay <i>et al.</i> , <i>PHAGE DISPLAY OF PEPTIDES AND PROTEINS: A LABORATORY MANUAL</i>

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	(Academic Press 1996). Thus, Claim 37 does not provide a patentable distinction over Claim 33. Accordingly, Claim 37 is obvious over the Count.
38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to $T_H 1$ cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon- $\gamma$ .	Claim 38 depends from Claim 33, which is obvious over the Count. Claim 38 simply adds that the immune response is differentiation of T cells to $T_H 1$ cells, and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides, and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon- $\gamma$ . It was well known that $T_H 1$ cells are derived from T cells and that $T_H 1$ cells produce IL-2 and IFN- $\gamma$ . See, e.g., Paul <i>et al.</i> , "Lymphocyte responses and cytokines," <i>Cell</i> 76: 241-251, 241-242 (1994); and Parronchi <i>et al.</i> , "IL-4 and IFN ( $\alpha$ and $\gamma$ ) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones," <i>J. Immunol.</i> 149(9): 2977-83 (1992). It was also commonly known that certain cytokines promote differentiation of T cells to $T_H 1$ cells and production of IL-2 and IFN- $\gamma$ . <i>Id.</i> See also, Mosmann <i>et al.</i> , "Heterogeneity of Cytokine Secretion Patterns and Functions of Helper T cells," <i>Adv. Immunol.</i> 46: 111-147 (1989). It would have been obvious to one skilled in the art to screen for cytokines encoded by the recombinant polynucleotides that induce T cells to produce IL-2 and IFN- $\gamma$ . Claim 38 does not patentably distinguish over Claim 33, which is obvious over the Count. Thus, Claim 38 is also obvious over the Count.

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<p><b>39.</b> The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.</p>	<p>Claim 39 depends from Claim 32, which is obvious over the Count. Claim 39 adds that the cytokine encoded by the optimized polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the polynucleotide into a mammal, and determining whether an immune response is induced against the cytokine. It was well known that cytokines were involved in mediating immune responses and that such immune responses could be readily measured. <i>See, e.g., Paul et al., "Lymphocyte responses and cytokines," Cell 76: 241-251, 241-242 (1994); Parronchi et al., "IL-4 and IFN (<math>\alpha</math> and <math>\gamma</math>) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones," J. Immunol. 149(9): 2977-83 (1992); and HUMAN CYTOKINES: HANDBOOK FOR BASIC AND CLINICAL RESEARCH, Vol. II (Aggarwal &amp; Gutterman eds. 1996).</i> It would have been obvious to one skilled in the art to screen for a cytokine that induces a reduced immune response compared to the immune response induced by a cytokine encoded by a non-optimized polynucleotide by introducing the cytokine of interest into a mammal and determining whether an immune response is induced. Thus, Claim 39 does not provide a patentable distinction over Claim 32, which is obvious over the Count. Accordingly, Claim 39 is obvious over the Count</p>
<p><b>40.</b> The method of claim 29, wherein the</p>	<p>Claim 40 depends from Claim 29, which is</p>

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co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the cell is tested for ability to costimulate an immune response.	anticipated by the Count. Claim 40 merely specifies that the co-stimulator is B7-1 (CD80) or B7-2 (CD86) and the cell is tested for ability to co-stimulate an immune response. B7-1, also known as CD80, and B7-2, also known as CD86, were well known co-stimulators. Hathcock <i>et al.</i> , "Comparative Analysis of B7-1 and B7-2 Costimulatory Ligands: Expression and Function," <i>J. Exp. Med.</i> 180: 631-40 (1994). Thus, this limitation does not provide a patentable distinction over Claim 29, which is anticipated by the Count. Accordingly, Claim 40 is obvious over the Count.
41. The method of any of claims 1, 3, or 5, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.	Claim 41 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 41 only adds that the optimized recombinant polynucleotide encodes a cytokine antagonist. Cytokine antagonists were well known and would have been an obvious choice to one skilled in the art. See, e.g., Paul <i>et al.</i> , "Lymphocyte responses and cytokines," <i>Cell</i> 76: 241-51, 241-2 (1994); Baggolini <i>et al.</i> , <i>Annu. Rev. Immunol.</i> 15: 675-705, 675 (1997); Greenfeder <i>et al.</i> , "Insertion of a Structural Domain of Interleukin (IL)-1 $\beta$ Confers Agonist Activity to the IL-1 Receptor Antagonist," <i>J. Biol. Chem.</i> 270(38): 22460-66 (1995); and Hannum <i>et al.</i> , "Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor," <i>Nature</i> 343(6256): 336-40 (1990). Claim 41 does not patentably distinguish over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 41 is obvious over the Count.

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42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective signal sequence.	Claim 42 depends from Claim 41, which is obvious over the Count. Claim 42 only adds that the cytokine antagonist is a soluble cytokine receptor or a transmembrane cytokine receptor having a defective signal sequence. That soluble cytokine receptors function as cytokine antagonists was well known and would have been an obvious choice to one skilled in the art. See, e.g., Curtis <i>et al.</i> , "Recombinant Soluble Interleukin-11 (IL-11) Receptor alpha Chain Can Act as an IL-11 Antagonist," <i>Blood</i> 90(11): 4403-12 (1997); and Alcami <i>et al.</i> , "A soluble receptor for interleukin-1 beta encoded by vaccinia virus: a novel mechanism of virus modulation of the host response to infection," <i>Cell</i> 71(1): 153-67 (1992). It was also well known that a transmembrane cytokine receptor comprises an extracellular domain, transmembrane domain, and intracellular domain, that the extracellular domains of such cytokine receptor binds the cytokine, and that the intracellular domain is typically involved in cytokine receptor signaling (see, e.g., JAK-STAT signaling pathway of the IFN- $\gamma$ receptor as discussed in Bach <i>et al.</i> , "The IFN gamma receptor: a paradigm for cytokine receptor signaling," <i>Annu. Rev. Immunol.</i> 15: 563-91 (1997)). It would have been obvious to the skilled artisan that a transmembrane cytokine receptor that binds the cytokine through its extracellular domain but has a defective signal sequence (e.g., defective intracellular domain) would function as a cytokine antagonist. Thus, Claim 42 does not patentably distinguish over Claim

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	41, which is obvious over the Count. Therefore, Claim 42 is obvious over the Count.
43. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of $\Delta$ IL-1 OR and $\Delta$ IL-4R.	Claim 43 depends from Claim 41, which is obvious over the Count. Claim 43 merely provides that the cytokine antagonist is $\Delta$ IL-10R or $\Delta$ IL-4R. It was well known that a deletion mutant of a cytokine receptor, e.g., comprising only the extracellular domain of the receptor, could function as a soluble cytokine antagonist. For example, it was known that a deletion mutant comprising the extracellular domain of IL-10 receptor functioned as an IL-10 antagonist. Tan <i>et al.</i> , "Characterization of recombinant extracellular domain of human interleukin-10 receptor." <i>J. Biol. Chem.</i> 270(21): 12906-11 (1995). It was also known that a deletion mutant of IL-4 functioned as an IL-4 antagonist. Atamas <i>et al.</i> , "An alternative splice variant of human IL-4, IL-4 delta 2, inhibits IL-4-stimulated T cell proliferation," <i>J. Immunol.</i> 156(2): 435-41 (1996). Thus, Claim 43 limitation does not patentably distinguish over Claim 41, which is obvious over the Count. Accordingly, Claim 43 is obvious over the Count.

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<b>44.</b> The method of any of claims 1, 3, or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T <sub>H</sub> 1 immune response.	<p>Claim 44 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. The only limitation added by Claim 44 is that the optimized polynucleotide encodes a polypeptide capable of inducing a predominantly T<sub>H</sub>1 immune response. It was well known that certain cytokines promote differentiation of T cells to predominantly T<sub>H</sub>1 cells. Paul <i>et al.</i>, "Lymphocyte responses and cytokines," <i>Cell</i> 76: 241-251, 241-242 (1994); and Mosmann <i>et al.</i>, "Heterogeneity of Cytokine Secretion Patterns and Functions of Helper T cells," <i>Adv. Immunol.</i> 46: 111-147 (1989). It would have been obvious to one skilled in the art to screen for polynucleotides that encode polypeptides capable of inducing a predominantly T<sub>H</sub>1 immune response. Claim 44 does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 44 is obvious over the Count.</p>
<b>45.</b> The method of any of claims 1, 3, or 5 wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T <sub>H</sub> 2 immune response.	<p>Claim 45 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. The only limitation added by Claim 45 is that the optimized polynucleotide encodes a polypeptide capable of inducing a predominantly T<sub>H</sub>2 immune response. It was well known that certain immunomodulatory polynucleotides encode polypeptides (<i>e.g.</i>, cytokines) that promote differentiation of T cells to predominantly T<sub>H</sub>2 cells. Paul <i>et al.</i>, "Lymphocyte responses and cytokines," <i>Cell</i> 76: 241-51, 241-2 (1994); and Mosmann <i>et al.</i>, "Heterogeneity of Cytokine Secretion Patterns and Functions of Helper T cells," <i>Adv.</i></p>

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	<p><i>Immunol.</i> 46: 111-147 (1989). It would have been obvious to one skilled in the art to screen for polynucleotides that encode polypeptides capable of inducing a predominantly T<sub>H</sub>2 immune response. This limitation does not patentably distinguish over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 45 is obvious over the Count.</p>
<p>46. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response;</p> <p>whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host;</p> <p>and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.</p>	<p>Claim 46 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. As each of the whereby clauses is written in optional language, Claim 46 merely adds that the optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response. It would have been obvious to one skilled in the art to screen the library of polynucleotides (or the respective polypeptides encoded therefrom) for those that exhibit a decrease in an unwanted modulatory effect on an immune response in a recipient (e.g., a polypeptide that has a decreased ability to elicit an immune response in a recipient or exhibits decreased antigenicity in a recipient) compared to a parental polynucleotide (or polypeptide encoded therefrom) from which the library was created. For example, specific allergens have long been subcutaneously administered in increasing amounts to allergy patients for treatment of allergies. In such protocols, the allergens serve as immunomodulators to modulate allergic immune responses. However, specific immunotherapy of allergies using natural allergen antigens carries a risk of inducing IgE-</p>

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	<p>mediated anaphylaxis. It was well known that mutant allergens having a decreased antigenicity compared to the wild-type allergen, and thereby having decreased ability to induce IgE-mediated allergic responses, could be made by mutagenesis methods. <i>See, e.g.,</i> Dudler <i>et al.</i>, "A Link Between Catalytic Activity, IgE-Independent Mast Cell Activation and Allergenicity of Bee Venom Phospholipase A<sub>2</sub>," <i>J. Immunol.</i> 155(5): 2605-13 (1995); and Noguchi <i>et al.</i>, "IgE Responsiveness to <i>Dermatophagoides farinae</i> in Young Asthmatic Children: IgE Binding Study Using Recombinant Allergens of Der f1, Der f2 and Mutant Proteins of Der f2," <i>Int. Arch. Allergy Immunol.</i> 110(4): 380-7 (1996). Various methods for measuring the level of humoral and/or cellular immune responses also were well known. <i>See, e.g.,</i> Sambrook <i>et al.</i>, MOLECULAR CLONING: A LABORATORY MANUAL (1989), <i>supra</i>. Thus, Claim 46 does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 46 is obvious over the Count.</p>
<p>47. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response;</p> <p>whereby application of the method can be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said</p>	<p>Claim 47 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. As each of the whereby clauses is written in optional language, Claim 47 merely adds that the optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response. It would have been obvious to one skilled in the art to screen the library of polynucleotides (or the respective polypeptides encoded therefrom)</p>

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<p>molecule, where said recipient can be a human or an animal host;</p> <p>and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule.</p>	<p>for those that exhibit an increase in a desirable modulatory effect on an immune response (e.g., a polypeptide that has an increased ability to elicit an immune response or exhibits increased antigenicity in a recipient) compared to a parental polynucleotide (or polypeptide encoded therefrom) from which the library was created. Various methods for measuring the level of humoral and/or cellular immune responses also were well known. <i>See, e.g., Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL (1989), supra.</i> Therefore, Claim 47 does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 47 is obvious over the Count.</p>
<p><b>48.</b> The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response;</p> <p>whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as an increased ability to elicit a second immune response from a second host recipient of said molecule;</p> <p>whereby the first and the second</p>	<p>Claim 48 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. As each of the whereby clauses is written in optional language, Claim 48 only adds that the optimized modulatory effect is a decrease in a first unwanted modulatory effect on an immune response and an increase in a second desirable modulatory effect on an immune response. It would have been obvious to one skilled in the art to screen the library of polynucleotides (or the respective polypeptides encoded therefrom) for one or more having both a decrease in a first unwanted modulatory effect on an immune response and an increase in a second desirable modulatory effect on an immune response. For example, it would be obvious to seek an optimized polypeptide having a decreased antigenicity (<i>i.e.</i>, decrease in a first unwanted</p>

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<p>recipient hosts can be the same or different;          whereby each of the first and the second recipient hosts can be a human or an animal host;          and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.</p>	<p>effect) and an improved half-life (<i>i.e.</i>, increase in a second desirable modulatory effect) compared to a non-optimized polypeptide. Such polypeptide could result from, <i>e.g.</i>, alterations in the glycosylation sites of the optimized polypeptide compared to the non-optimized polypeptide. It was well known that certain changes in the amino acid sequence of a polypeptide can alter a polypeptide's glycosylation sites and that alterations in glycosylation sites can affect the antigenicity and/or half-life of the polypeptide. <i>See, e.g.</i>, WO 98/27230; WO 99/23107; and WO 99/41383. <i>See also</i>, the remarks for Claim 46 above. As this limitation does not provide a patentable distinction over Claim 1, 3 or 5, each of which is anticipated by the Count, Claim 48 also is obvious over the Count.</p>
<p><b>49.</b> The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector;          whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, and enhancer, a promoter, and operator, and an</p>	<p>Claim 49 depends from Claim 48, which is obvious over the Count. Claim 49 merely adds that the first and said second modulatory effect on an immune response are evolved for a first and a second module on the same multimodule vaccine vector. The module can be an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, and enhancer, a promoter, and operator, or an intron. Each of these components was well known and would have been obvious choices to a skilled artisan. Furthermore, it would have been obvious to a skilled artisan that such</p>

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intron.	<p>vector components could be evolved. <i>See, e.g.</i>, Donnelly <i>et al.</i>, "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-48, 620 (1997); Levinson, "Expression of Heterologous Genes in Mammalian Cells," <i>in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY</i> 185: 485-87 (David V. Goeddel ed., Acad. Press, Inc., 1990); Le Grice, "Regulated Promoter for High-Level Expression of Heterologous Genes for <i>Bacillus subtilis</i>," <i>in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY</i> 185: 201-14 (1990), <i>supra</i>; Kaufman, "Vectors Used for Expression in Mammalian Cells," <i>in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY</i> 185: 487-511 (1990), <i>supra</i>; Balbas <i>et al.</i>, "Design and Construction of Expression Plasmid Vectors in <i>Escherichia coli</i>," <i>in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY</i> 185: 14-37 1990, <i>supra</i>; and WO 99/41369. Thus, Claim 49 does not provide a patentable distinction over Claim 48, which is obvious over the Count. Accordingly, Claim 49 is obvious over the Count.</p>
<p><b>50.</b> The method of any of claims 1, 3, or 5, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby;</p> <p>whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of</p>	<p>Claim 50 depends from any of Claims 1, 3 or 5, each of which is anticipated by the Count. As each of the whereby clauses is written in optional language, Claim 50 merely adds the optimized modulatory effect on an immune response comprises an increase in the stability of the immunomodulatory polynucleotide or polypeptide encoded thereby. It would have been obvious to one of skill that an immunomodulatory polynucleotide or polypeptide encoded thereby that is not stable</p>

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<p>storage and/or length of time before expiration of activity upon storage;</p> <p>and whereby application of the method can also be used to generate a molecule having an increased stability <i>in vivo</i> upon administration to a host recipient, thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of elimination or destruction by the host recipient.</p>	<p>and thus rendered inactive under certain conditions (<i>e.g.</i>, by acid degradation) would have less ability to modulate an immune response than one that retains its activity under those conditions. It would also have been obvious to one of skill that improving the stability of an immunomodulatory polynucleotide or polypeptide encoded thereby under adverse conditions (<i>e.g.</i>, improving the molecule's ability to resist digestive acids generated <i>in vivo</i>) would improve the ability of that molecule to modulate the immune response under those conditions, since the molecule would retain its activity for a longer period. It was also known that recombinant DNA technology could be used to design mutant molecules having increased stability, while retaining their specific activity. <i>See, e.g.</i>, Villbrandt <i>et al.</i>, <i>Protein Eng'g</i> 10(11): 1281-88 (1997); WO 98/27230; WO 99/23107; WO 99/41369; and WO 99/41383. Claim 50 does not patentably distinguish over Claim 1, 3 or 5, each of which is anticipated by the Count. Thus, Claim 50 is obvious over the Count.</p>
<p><b>51.</b> The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient as compared with prior to optimization;</p> <p>whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipients.</p>	<p>Claim 51 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 51 simply adds that the immunomodulatory polynucleotide or polypeptide encoded thereby has an optimized modulatory effect on an immune response in a human recipient as compared with prior to optimization. The method can thus be used to generate an optimized genetic vaccine for humans. It was well known in the art that recombinant DNA technology could be used to</p>

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	create genetic vaccines for humans. <i>See, e.g.</i> , Donnelly <i>et al.</i> , "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-48, 620 (1997). Claim 51 does not patentably distinguish over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 51 is obvious over the Count.
<p><b>52.</b> The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient as compared with prior to optimization;</p> <p>whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not fanned or raised by man, domesticated animals, and non-domesticated animals.</p>	Claim 52 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 52 only adds that the immunomodulatory polynucleotide or polypeptide encoded thereby has an optimized modulatory effect on an immune response in an animal recipient as compared with prior to optimization, and the method can thus be used to generate an optimized genetic vaccine for animal recipients. Animals included are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals. It was well known in the art that recombinant DNA technology could be used to create genetic vaccines for animals. <i>See, e.g.</i> , Donnelly <i>et al.</i> , "DNA Vaccines," <i>Annu. Rev. Immunol.</i> 15: 617-48, 620 (1997); and Schrijver <i>et al.</i> , "Comparison of DNA application methods to reduce BRSV shedding in cattle," <i>Vaccine</i> 16(2-3): 130-4 (1998). Claim 52 does not patentably distinguish over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 52 is obvious over the Count.
<p><b>53.</b> A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising:</p>	Claim 53 is an independent claim. The method of Claim 53 is an obvious extension of at least the method of Claim 5, which is anticipated by the Count. Claim 53 is directed to a method for obtaining an optimized polynucleotide that

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<p>a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and</p> <p>b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly;</p> <p>whereby application of the method can thus be used to generate an optimized molecule for human recipients &amp;/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals;</p> <p>whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner;</p> <p>whereby these directed evolution methods include the introduction of point</p>	<p>encodes an accessory molecule that improves the transport or presentation of antigens by a cell. The method comprises creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule and screening the library to identify an optimized non-stochastically generated polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on the surface of the cell compared to an accessory molecule encoded by a polynucleotide not subjected to non-stochastic reassembly. The only substantive difference between Claims 53 and 5 is that Claim 53 specifies an optimized non-stochastically generated polynucleotide that encodes an accessory molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on its surface, while Claim 5 specifies an optimized immunomodulatory polynucleotide that encodes a polypeptide that has an enhanced ability to modulate an immune response. However, it would have been obvious to a skilled artisan that a polypeptide that has enhanced ability to modulate an immune response (as in Claim 5) would include a polypeptide (<i>i.e.</i>, accessory molecule) that confers upon a cell an increased or decreased ability to transport or present an antigen (as in Claim 53). For example, it would have been obvious to a skilled artisan that increasing the ability of a cell to present an antigen on its</p>

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<p>mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein;</p> <p>and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.</p>	<p>surface would increase the immune response that could be induced, because more effective antigen presentation by the cell would induce a greater immune response. It would also have been obvious that decreasing the ability of a cell to present an antigen on its surface would decrease the immune response that could be induced, because less effective antigen presentation by the cell would induce less of an immune response. Similarly, it would have been obvious that increasing a cell's ability to transport an antigen to its surface could increase the resultant immune response, because more effective transportation of the antigen to the cell surface would likely result in more effective antigen presentation and stimulation of a greater immune response. Claim 53 does not provide a patentable distinction over at least Claim 5, which is anticipated by the Count. Thus, Claim 53 is obvious over the Count.</p>
<p><b>54.</b> The method of claim 53, wherein the screening involves:</p> <p>i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and</p> <p>ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.</p>	<p>Claim 54 depends from Claim 53, which is obvious over the Count. Compared to Claim 53, Claim 54 only adds that the screening involves introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors, introducing the library of vectors into mammalian cells, and identifying those cells that exhibit increased or decreased immunogenicity to the antigen. Methods for introducing polynucleotides into vectors (including, e.g., vectors that encode an antigen), methods for introducing such vectors into mammalian cells for expression of the</p>

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	polynucleotides, and methods for identifying cells that exhibit increased or decreased immunogenicity to the expressed antigen were well known. <i>See, e.g.</i> , Sambrook <i>et al.</i> , MOLECULAR CLONING: A LABORATORY MANUAL (1989), <i>supra</i> . Claim 54 does not patentably distinguish over at least Claim 53 or Claim 5, each of which is respectively obvious over or anticipated by the Count. Thus, Claim 54 is obvious over the Count.
55. The method of claim 53, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.	Claim 55 depends from Claim 53, which is obvious over the Count. Claim 55 merely adds that the accessory molecule comprises a proteasome or a TAP polypeptide. Proteasomes were well known to be involved in antigen presentation and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i> , Gaczynska <i>et al.</i> , "Proteasome subunits X and Y alter peptidase activities in opposite ways to the interferon-gamma-induced subunits LMP2 and LMP7," <i>J. Biol. Chem.</i> 271(29): 17275-80 (1996); Groettrup <i>et al.</i> , "The subunits MECL-1 and LMP2 are mutually required for incorporation in the 20S proteasome," <i>PNAS USA</i> 94: 8970-75 (1997); and Stohwasser <i>et al.</i> , "Molecular cloning of the mouse proteasome subunits MC14 and MECL-1: reciprocally regulated tissue expression of interferon-gamma-modulated proteasome subunits," <i>Eur. J. Immunol.</i> 27(5): 1182-7 (1997). TAP polypeptides were also well known to be involved in antigen presentation and transport and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i> , York <i>et al.</i> , "Antigen processing and presentation by the class I major

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	<p>histocompatibility complex," <i>Annu. Rev. Immunol.</i> 14: 369-96 (1996). Claim 55 does not provide a patentable distinction over Claim 53, which is obvious over the Count. Accordingly, Claim 55 is obvious over the Count.</p>
<p><b>56.</b> The method of claim 53, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.</p>	<p>Claim 56 depends from Claim 53, which is obvious over the Count. Claim 56 merely adds that the accessory molecule comprises a cytotoxic T-cell inducing sequence. It was well known that certain peptides induce stimulation of cytotoxic T cells. <i>See, e.g.,</i> York <i>et al.</i>, "Antigen processing and presentation by the class I major histocompatibility complex," <i>Annu. Rev. Immunol.</i> 14: 369-96 (1996). Furthermore, it was well known that certain proteins are better able than others to carry MHC class I epitopes, because they are more readily used by the cellular machinery involved in the necessary processing for class I epitope presentation. It was well known that certain cytotoxic T-cell inducing sequences can be used to carry heterologous class I epitopes for the purpose of vaccinating against the pathogen from which the heterologous epitopes are derived. For example, it was known that hepatitis B surface antigen (HBsAg) induces a cytotoxic T cell response and can be used to carry heterologous epitopes from another pathogenic protein to improve the presentation of epitopes of that pathogenic protein. <i>See, e.g.,</i> Fomsgaard <i>et al.</i>, "Improved humoral and cellular immune response against the gp120 V3 loop of HIV-1 following genetic immunization with a chimeric DNA vaccine encoding the V3</p>

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	<p>inserted in the hepatitis B surface antigen," <i>Scand. J. Immunol.</i> 47(4): 289-95 (1998); Le Borgne <i>et al.</i>, "In Vivo Induction of Specific Cytotoxic T Lymphocytes in Mice and Rhesus Macaques Immunized with DNA Vector Encoding an HIV Epitope Fused with Hepatitis B Surface Antigen," <i>Virology</i> 240: 304-15 (1998); and Eckhart <i>et al.</i>, "Immunogenic presentation of a conserved gp41 epitope of human immunodeficiency virus type 1 on recombinant surface antigen of hepatitis B virus," <i>J. Gen. Virol.</i> 77 (9): 2001-8 (1996). Thus, it would have been obvious to a skilled artisan to use cytotoxic T-cell inducing sequences in the method of Claim 53 for purposes of improving antigen presentation. Accordingly, Claim 56 is obvious over the Count.</p>
57. The method of claim 56, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.	Claim 57 depends from Claim 56, which is obvious over the Count. Claim 57 merely adds that the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen. As discussed for Claim 56, it was well known that hepatitis B surface antigen induces a cytotoxic T cell response and can be used to carry heterologous epitopes from another pathogenic protein to improve the presentation of epitopes of that pathogenic protein. Thus, it would have been obvious to a skilled artisan to use such cytotoxic T-cell inducing sequence for purposes of improving antigen presentation. Claim 57 does not patentably distinguish over Claim 56, which is obvious over the Count. Accordingly, Claim 57 is obvious over the Count.

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<p><b>58.</b> The method of claim 53, wherein the accessory molecule comprises an immunogenic agonist sequence.</p>	<p>Claim 58 depends from Claim 53, which is obvious over the Count. The only limitation that is added by Claim 58 is that the accessory molecule comprises an immunogenic agonist sequence. This limitation does not provide a patentable distinction over Claim 53. For example, it was well known in the art that one can generate immunogenic agonist sequences, including e.g., those that enhance T cell activation. See, e.g., Zaremba <i>et al.</i>, "Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carinoembryonic antigen," <i>Cancer Res.</i> 57(20): 4570-77 (1997); Fomsgaard <i>et al.</i>, <i>Scand. J. Immunol.</i> 47(4): 289-95 (1998); and Le Borgne <i>et al.</i>, <i>Virology</i> 240: 304-15 (1998). It was also well known in the art that such immunogenic agonist sequences could be used as accessory molecules to enhance antigen presentation. See, e.g., Fomsgaard <i>et al.</i>, Le Borgne <i>et al.</i>, and Eckhart <i>et al.</i>, and remarks for Claims 56 and 57, <i>supra</i>. It would have been obvious to a skilled artisan to use immunogenic agonist sequences in the method of Claim 53 for purposes of improving antigen presentation. As this limitation does not patentably distinguish over Claim 53, which is obvious over the Count, Claim 58 also is obvious over the Count.</p>
<p><b>59.</b> A method for obtaining an immunomodulatory polynucleotide that has, an optimized expression in a recombinant expression host, the method comprising:  creating a library of non-stochastically</p>	<p>Claim 59 is an independent claim. Claim 59 is an obvious variation of at least Claim 1, which is anticipated by the Count. Claim 59 provides a method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host. The method</p>

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<p>generated progeny polynucleotides from a parental polynucleotide set;</p> <p>whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner;</p> <p>whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein;</p> <p>and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.</p>	comprises creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner. The directed evolution methods include the introduction of mutations by non-stochastic methods, including by gene site saturation mutagenesis and synthetic ligation polynucleotide reassembly. The steps of the method of Claim 59 are analogous to the steps of the method of Claim 1. The only substantive difference between Claims 59 and 1 is that Claim 59 specifies an immunomodulatory polynucleotide that exhibits optimized expression in a recombinant expression host, while Claim 1 specifies an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response. However, it would have been obvious to a skilled artisan that an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response would include an immunomodulatory polynucleotide that has an optimized expression in the host. For example, it would have been obvious to a skilled artisan that the better an immunomodulatory polynucleotide is able to be expressed in a host, the better that immunomodulatory polynucleotide would be able to modulate the immune response in the host. It would have been obvious that an immunomodulatory polynucleotide whose

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	<p>expression in the host is optimized would have a greater effect on the immune response than an immunomodulatory polynucleotide whose expression is not optimized. Moreover, a variety of methods for determining the expression of a polynucleotide in a host were well known. For example, flow cytometry-based screening methods (FACS) were well known methods used for identification of cells that expressed a desired polynucleotide on the cell surface and for sorting of cells for different levels of expression of the polynucleotide. Other methods for measuring expression of a polynucleotide in a recombinant expression host known included ELISA assays using supernatants of the transfected host cells and Western blots. <i>See, e.g., Dagan et al., "High level expression and production of recombinant human interleukin analogs," Protein Exp. Purif.</i> 3(4): 290-4 (1992). Thus, Claim 59 is merely an obvious variation of at least Claim 1, which is anticipated by the Count. Accordingly, Claim 59 is obvious over the Count.</p>
<p><b>60.</b> A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising:</p> <p>screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide</p>	<p>Claim 60 is an independent claim. Claim 60 is an obvious variation of at least Claim 3, which is anticipated by the Count. The steps of the method of Claim 60 are analogous to the steps of the method of Claim 3. The only substantive difference between Claims 60 and 3 is that Claim 60 specifies that the optimized immunomodulatory polynucleotide has optimized expression in a host, while Claim 3 specifies that the optimized immunomodulatory polynucleotide exhibits an enhanced ability to modulate an immune response. However, it</p>

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from which the library was created.	would have been obvious to a skilled artisan that an immunomodulatory polynucleotide that has an enhanced ability to modulate an immune response would include an immunomodulatory polynucleotide that has optimized expression in the host. For example, it would have been obvious to a skilled artisan that the better an immunomodulatory polynucleotide is able to be expressed in a host, the better that immunomodulatory polynucleotide would be able to modulate the immune response in the host. It would have been obvious to one of skill that an immunomodulatory polynucleotide whose expression in the host is optimized would have a greater effect on the immune response than an immunomodulator whose expression is not optimized. In addition, as discussed for Claim 59, methods for determining the expression of a polynucleotide in a host were well known. <i>See, e.g.,</i> Dagan <i>et al.</i> , "High level expression and production of recombinant human interleukin analogs," <i>Protein Expr. Purif.</i> 3(4): 290-4 (1992). Thus, Claim 60 is obvious over the Count because it is simply an apparent variation of at least Claim 3, which is anticipated by the Count.
61. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising:  a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and	Claim 61 is an independent claim. Claim 61 is an obvious variation of at least Claim 5, which is anticipated by the Count. The steps of the method of Claim 61 are analogous to the steps of the method of Claim 5. The only substantive difference between Claims 61 and 5 is that Claim 61 specifies an immunomodulatory polynucleotide that has optimized expression in the host, while Claim 5 specifies an

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<p>b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created;</p> <p>whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner;</p> <p>whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein;</p> <p>and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.</p>	<p>immunomodulatory polynucleotide that has an enhanced ability to modulate an immune response. It would have been obvious to one skilled in the art that an immunomodulatory polynucleotide that has an enhanced ability to modulate an immune response in a host would include an immunomodulatory polynucleotide that has optimized expression in the host. For example, it would have been obvious to a skilled artisan that the better an immunomodulatory polynucleotide is able to be expressed in a host, the better that immunomodulatory polynucleotide would be able to modulate the immune response in the host. It would have been obvious to one of skill that an immunomodulatory polynucleotide whose expression in the host is optimized would have a greater effect on the immune response than an immunomodulator whose expression in the host is not optimized. Moreover, as discussed for Claim 59, methods for determining the expression of a polynucleotide in a host were well known. Thus, Claim 61 is merely an obvious extension of at least Claim 5, which is anticipated by the Count. Accordingly, Claim 61 is obvious over the Count.</p>
<p><b>62.</b> The method of any of claims 59-61, wherein the recombinant expression host is a prokaryote.</p>	<p>Claim 62 depends from any of Claims 59-61, each of which is obvious over the Count. Claim 62 merely adds that the recombinant expression host is a prokaryote. Prokaryotes were well known expression hosts. <i>See, e.g.,</i></p>

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	Le Grice, "Regulated Promoter for High-Level Expression of Heterologous Genes for <i>Bacillus subtilis</i> ," in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY 185: 201-14 (1990), <i>supra</i> ; and Balbas <i>et al.</i> , "Design and Construction of Expression Plasmid Vectors in <i>Escherichia coli</i> ," in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY 185: 14-37 (1990), <i>supra</i> . Specifying that the recombinant expression host is a prokaryote does not provide a patentable distinction over Claim 59, 60 or 61, each of which is obvious over the Count. Accordingly, Claim 62 is obvious over the Count.
63. The method of any of claims 59-61, wherein the recombinant expression host is a eukaryote.	Claim 63 depends from any of Claims 59-61, each of which is obvious over the Count. Claim 63 simply adds that the recombinant expression host is a eukaryote. Eukaryotes were well known expression hosts. See, e.g., Levinson, "Expression of Heterologous Genes in Mammalian Cells," in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY. 185: 485-87 (1990), <i>supra</i> ; Kaufman, "Vectors Used for Expression in Mammalian Cells," in METHODS IN ENZYMOLOGY: GENE EXPRESSION TECHNOLOGY 185: 487-511 (1990), <i>supra</i> ; and Tuite, "Strategies for the genetic manipulation of <i>Saccharomyces cerevisiae</i> ," <i>Crit. Rev. Biotechnol.</i> 12(1-2): 157-88 (1992). Specifying that the recombinant expression host is a eukaryote does not provide a patentable distinction over Claims 59-61, each of which is obvious over the Count. Accordingly, Claim 63 is obvious over the Count.

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64. The method of claim 63, wherein the recombinant expression host is a plant.	Claim 64 depends from Claim 63, which is obvious over the Count. Claim 64 merely adds that the recombinant expression host is a plant. Plants were well known expression hosts. <i>See, e.g.</i> , Miele, "Plants as bioreactors for biopharmaceuticals: regulatory considerations," <i>Trends Biotechnol.</i> 15(2): 45-50 (1997); and Ma <i>et al.</i> , "Antibody production and engineering in plants," <i>Ann. NY Acad. Sci.</i> 792: 72-81 (1996). Specifying that the recombinant expression host is a plant does not provide a patentable distinction over Claim 63, which is obvious over the Count. Accordingly, Claim 64 is obvious over the Count.
65. The method of claim 64, wherein the recombinant expression host is a monocot.	Claim 65 depends from Claim 64, which is obvious over the Count. Claim 65 merely designates that the plant is a monocot. Monocots were well known expression hosts. <i>See, e.g.</i> , Yoshie <i>et al.</i> , "Novel lymphocyte-specific CC chemokines and their receptors," <i>J. Leukoc. Biol.</i> 62(5): 634-44 (1997); and Fromm <i>et al.</i> , "Expression of genes transferred into monocot and dicot plant cells by electroporation." <i>PNAS USA</i> 82(17): 5824-8 (1985). Specifying that the recombinant plant expression host is a monocot does not provide a patentable distinction over Claim 64, which is obvious over the Count. Accordingly, Claim 65 is obvious over the Count.
66. The method of claim 64, wherein the recombinant expression host is a dicot.	Claim 66 depends from Claim 64, which is obvious over the Count. Claim 66 merely provides that the plant is a dicot. Dicots were well known expression hosts. <i>See, e.g.</i> , Yoshie <i>et al.</i> , "Novel lymphocyte-specific CC

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	chemokines and their receptors," <i>J. Leukoc. Biol.</i> 62(5): 634-44 (1997); and Fromm <i>et al.</i> , "Expression of genes transferred into monocot and dicot plant cells by electroporation." <i>PNAS USA</i> 82(17): 5824-8 (1985). Specifying that the recombinant plant expression host is a dicot does not provide a patentable distinction over Claim 64, which is obvious over the Count. Accordingly, Claim 66 is obvious over the Count.
67. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.	Claim 67 depends from any of Claims 1, 3, or 5 each of which is anticipated by the Count. Claim 67 further depends from any of Claims 53 or 59-61, each of which is obvious over the Count. Claim 67 merely adds that the library of non-stochastically generated progeny polynucleotides is created from a parental polynucleotide set by subjecting the parental polynucleotide set to gene site saturation mutagenesis. Gene site saturation mutagenesis was well known in the art and would have been an obvious choice to a skilled artisan. See, e.g., Hill <i>et al.</i> , "Mutagenesis with Degenerate Oligonucleotides: An Efficient Method for Saturating a Defined DNA Region with Base Pair Substitutions," IN METHODS IN ENZYMOLOGY RECOMBINANT DNA 155: 558-68 (Ray Wu, ed. Acad. Press Inc., 1987); Goff <i>et al.</i> , "Laboratory Methods: Efficient Saturation Mutagenesis of a Pentapeptide Coding Sequence Using Mixed Oligonucleotides," <i>DNA</i> 6(4): 381-8 (1987); and Murray <i>et al.</i> , "Saturation mutagenesis of a major histocompatibility complex protein domain: Identification of a single conserved

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	<p>amino acid important for allore cognition," <i>PNAS USA</i> 85:3535-39 (1988). Thus, Claim 67 does not patentably distinguish over any of Claims 1, 3, 5, 53, and 59-61. Accordingly, Claim 67 is obvious over the Count.</p>
<p><b>68.</b> The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.</p>	<p>Claim 68 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 68 also depends from any of Claims 53 or 59-61, each of which is obvious over the Count. Claim 68 merely adds that the library of non-stochastically generated progeny polynucleotides is created from a parental polynucleotide set by subjecting the parental polynucleotide set to synthetic ligation polynucleotide reassembly. Synthetic ligation polynucleotide reassembly was well known in the art at the time the Short '052 application was filed and would have been an obvious choice to a skilled artisan at the time. <i>See, e.g.</i>, U.S. Pat. No. 6,376,246. Thus, Claim 68 does not patentably distinguish over any of Claims 1, 3, 5, 53, and 59-61, in view of the state of the art at the time of filing. Accordingly, Claim 68 is obvious over the Count.</p>
<p><b>69.</b> The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.</p>	<p>Claim 69 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 69 also depends from any of Claims 53 or 59-61, each of which is obvious over the Count. Claim 69 merely adds that the library of non-stochastically generated progeny polynucleotides is created from a parental polynucleotide set by subjecting the parental polynucleotide set to gene site saturation mutagenesis and synthetic ligation</p>

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	<p>polynucleotide reassembly. Gene site saturation mutagenesis was well known at the time the Short '052 application was filed and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i>, references cited for Claim 67. Synthetic ligation polynucleotide reassembly was also well known in the art at the time the Short '052 application was filed and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i>, U.S. Pat. No. 6,376,246. Thus, Claim 69 does not provide a patentable distinction over any of Claims 1, 3, 5, 53, and 59-61, in view of the state of the art at the time of filing. Accordingly, Claim 69 is obvious over the Count.</p>
70. The method of claim 1, wherein the directed evolution method is synthetic ligation reassembly.	<p>Claim 70 depends from Claim 1, which is anticipated by the Count. Claim 70 merely adds that the directed evolution method is synthetic ligation reassembly. Synthetic ligation reassembly was well known in the art at the time the Short '052 application was filed and would have been an obvious choice to a skilled artisan at the time. <i>See, e.g.</i>, U.S. Pat. No. 6,376,246. Thus, Claim 70 does not provide a patentable distinction over Claim 1, which is anticipated by the Count, in view of the state of the art at the time of filing. Accordingly, Claim 70 is obvious over the Count.</p>
71. The method of claim 1, wherein the directed evolution method is gene site saturated mutagenesis.	<p>Claim 71 depends from Claim 1, which is anticipated by the Count. Claim 71 merely adds that the directed evolution method is gene site saturated mutagenesis. Gene site saturated mutagenesis was well known and thus does not</p>

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	provide a patentable distinction over Claim 1, which is anticipated by the Count. <i>See, e.g.</i> , references cited for Claim 67. Accordingly, Claim 71 is obvious over the Count.
72. The method of claim 1, wherein the directed evolution method is non-stochastic ligation reassembly.	Claim 72 depends from Claim 1, which is anticipated by the Count. Claim 72 merely adds that the directed evolution method is non-stochastic ligation reassembly. Non-stochastic ligation reassembly was well known in the art at the time the Short '052 application was filed and thus does not provide a patentable distinction over Claim 1, which is anticipated by the Count, in view of the state of the art at the time of filing. <i>See, e.g.</i> , U.S. Pat. No. 6,376,246. Accordingly, Claim 72 is obvious over the Count.
73. The method of claim 1, wherein the directed evolution method is exonuclease-mediated reassembly.	Claim 73 depends from Claim 1, which is anticipated by the Count. Claim 73 merely adds that the directed evolution method is exonuclease-mediated reassembly. It was well known that nucleotide fragments could be generated using exonucleases and that nucleotide fragments could be reassembled to generate evolved polypeptides having improved properties. <i>See, e.g.</i> , Ostermeier <i>et al.</i> , "Combinatorial protein engineering by incremental truncation," <i>PNAS USA</i> 96: 3562-67 (1999); Ostermeier <i>et al.</i> , "A combinatorial approach to hybrid enzymes independent of DNA homology," <i>Nature</i> 19: 1205-09 (1999); WO 99/23107; and Sayers <i>et al.</i> , "5'-3' Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis," <i>Nucleic Acids Res.</i> 16: 791-802 (1988). Thus, this

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	limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 73 is obvious over the Count.
74. The method of claim 1, wherein the directed evolution method is end selection.	Claim 74 depends from Claim 1, which is anticipated by the Count. Claim 74 merely adds that the directed evolution method is end selection. The '258 patent describes a "method for selecting a subset of polynucleotides from a starting set of polynucleotides, which method is based on the ability to discriminate one or more selectable features (or selection markers) present anywhere in a working polynucleotide, so as to allow one to perform selection for (positive selection) &/or against (negative selection) each selectable polynucleotide . . . [E]nd-selection . . . is based on the use of a selection marker located in part or entirely in a terminal region of a selectable polynucleotide." '258 patent, Col. 358, l. 65 to Col. 259, l. 10. Selection markers include a polynucleotide sequence, polypeptide sequence, or any biological or biochemical tag, including markers that can be selected using methods based on the detection of radioactivity, enzymatic activity, fluorescence, any optical feature, magnetic property (e.g., magnetic beads), immunoreactivity, and hybridization. '258 patent, Col. 359, ll. 15-25. The use of such selection markers for such purposes was well known. See, e.g., WO 98/27230; Sambrook <i>et al.</i> , MOLECULAR CLONING: A LABORATORY MANUAL (1989), <i>supra</i> . Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated

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	by the Count. Accordingly, Claim 74 is obvious over the Count.
75. The method of claim 1, wherein the directed evolution method is shuffling.	Claim 75 depends from Claim 1, which is anticipated by the Count. Claim 75 only adds that the directed evolution method is shuffling. Shuffling was well known. Thus, the limitation does not provide a patentable distinction over Claim 1 in view of the state of the art. <i>See, e.g.,</i> Stemmer, "Searching Sequence Space," <i>Biotechnology</i> 13: 549-53 (1995); and Crameri <i>et al.</i> , "DNA Shuffling of a Family of Genes From Diverse Species Accelerates Directed Evolution," <i>Nature</i> 391: 288-91 (1998). Accordingly, Claim 75 is obvious over the Count.
76. The method of claim 1, wherein the immunomodulatory polynucleofide [sic] encodes a cancer antigen.	Claim 76 depends from Claim 1, which is anticipated by the Count. Claim 76 merely adds that the immunomodulatory polynucleotide encodes a cancer antigen. Cancer antigens were well known and would have been an obvious target. <i>See, e.g.,</i> Becket <i>et al.</i> , "Characterization of a Prostate Carcinoma Mucin-Like Antigen (PMA)," <i>Int. J. Cancer</i> 62: 703-10 (1995); and Apostolopoulos <i>et al.</i> , "Breast cancer immunotherapy: Current status and future prospects," <i>Immunol. and Cell. Biol.</i> 74: 457-64 (1996). Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 76 is obvious over the Count.
77. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a bacterial antigen.	Claim 77 depends from Claim 1, which is anticipated by the Count. Claim 77 merely adds that the immunomodulatory

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	<p>polynucleotide encodes a bacterial antigen. Bacterial antigens were well known and would have been an obvious target. <i>See, e.g.</i>, Censi <i>et al.</i>, "cag, a pathogenicity island of <i>Helicobacter pylori</i>, encodes type I-specific and disease-associated virulence factors," <i>PNAS USA</i> 93: 14648-53 (1996); and Hess <i>et al.</i>, "Superior efficacy of secreted over somatic antigen display in recombinant <i>Salmonella</i> vaccine induced protection against listeriosis," <i>PNAS USA</i> 93: 1458-63 (1996). Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 77 is obvious over the Count.</p>
78. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a viral antigen.	<p>Claim 78 depends from Claim 1, which is anticipated by the Count. Claim 78 merely adds that the immunomodulatory polynucleotide encodes a viral antigen. Viral antigens were well known in the art and would have been an obvious target. <i>See, e.g.</i>, Mattion <i>et al.</i>, "Characterization of recombinant polioviruses expressing regions of rotavirus VP4, hepatitis B surface antigen, and herpes simplex virus type 2 glycoprotein D," <i>J. Virol.</i> 69: 5132-37 (1995). Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 78 is obvious over the Count.</p>
79. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a parasite antigen.	<p>Claim 79 depends from Claim 1, which is anticipated by the Count. Claim 79 simply adds that the immunomodulatory polynucleotide encodes a parasite antigen.</p>

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	Parasite antigens were well known in the art and would have been an obvious target. <i>See, e.g.</i> , Beck <i>et al.</i> , "Analysis of Multiple Plasmodium falciparum Infections in Tanzanian Children during the Phase II Trial of Malaria Vaccine SPf66," <i>J. Inf. Disease</i> 175: 921-26 (1997). Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 79 is obvious over the Count.
<b>80.</b> The method of claim 1, wherein the immunomodulatory polynucleotide encodes a self-antigen.	Claim 80 depends from Claim 1, which is anticipated by the Count. Claim 80 only adds that the immunomodulatory polynucleotide encodes a self-antigen. Self-antigens were well known in the art and would have been an obvious target. <i>See, e.g.</i> , Quaratino <i>et al.</i> , "Similar antigenic surfaces, rather than sequence homology dictate T-cell epitope molecular mimicry," <i>PNAS USA</i> 92: 10398-402 (1995); and Zaremba <i>et al.</i> , "Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen," <i>Cancer Res.</i> 57(20): 4570-77 (1997). Thus, this limitation does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 80 is obvious over the Count.
<b>81.</b> The method of claim 1, wherein the immune response is a humoral immune response.	Claim 81 depends from Claim 1, which is anticipated by the Count. Claim 81 simply adds that the immune response is a humoral immune response. It was well known that a humoral response was one type of immune response. Paul, <i>The Immune System: An Introduction</i> , Chap. 1, pp. 1-20 in

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	FUNDAMENTAL IMMUNOLOGY (W. E. Paul. New York, Raven Press, 1993); and Ciernik <i>et al.</i> , "Induction of Cytotoxic T Lymphocytes and Antitumor Immunity with DNA Vaccines Expressing Single T Cell Epitopes," <i>J. Immunol.</i> 156: 2369-75 (1996). Merely specifying that the immune response is a humoral response does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 81 is obvious over the Count.
82. The method of claim 1, wherein the immune response is a cellular immune response.	Claim 82 depends from Claim 1, which is anticipated by the Count. Claim 82 simply adds that the immune response is a cellular immune response. It was well known that a cellular response was one type of immune response. Paul, <i>The Immune System: An Introduction</i> , Chap. 1, pp. 1-20 in FUNDAMENTAL IMMUNOLOGY (W. E. Paul. New York, Raven Press, 1993); and Ciernik <i>et al.</i> , "Induction of Cytotoxic T Lymphocytes and Antitumor Immunity with DNA Vaccines Expressing Single T Cell Epitopes," <i>J. Immunol.</i> 156: 2369-75 (1996). Merely specifying that the immune response is a cellular response does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 82 is obvious over the Count.
83. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a cytokine.	Claim 83 depends from Claim 1, which is anticipated by the Count. Claim 83 simply adds that the immunomodulatory polynucleotide encodes a cytokine. Cytokines were well known and would have been obvious

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	targets to a skilled artisan. <i>See, e.g.</i> , HUMAN CYTOKINES: HANDBOOK FOR BASIC AND CLINICAL RESEARCH, Vol. II (Aggarwal & Guterman eds. 1996); and Paul <i>et al.</i> , <i>Cell</i> 76: 241-51 (1994). Merely specifying that the immunomodulatory polynucleotide is a cytokine does not provide a patentable distinction over Claim 1, which is anticipated by the Count. Accordingly, Claim 83 is obvious over the Count.
84. The method of claim 83, wherein the cytokine is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14 [sic], IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , or IL-20 (MDA-7).	Claim 84 depends from Claim 83, which is obvious over the Count. Claim 84 merely adds that the cytokine is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF- $\alpha$ , IFN- $\alpha$ , IFN- $\gamma$ , or IL-20 (MDA-7). Each of these cytokines was well known and would have been an obvious choice to a skilled artisan. <i>See, e.g.</i> , the references discussed for Claim 33. Merely specifying a known cytokine does not patentably distinguish over Claim 83, which is obvious over the Count. Thus, Claim 84 is obvious over the Count.
85. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined <i>in vitro</i> .	Claim 85 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 85 merely adds that the immune response prior to optimization or following optimization is determined <i>in vitro</i> . Methods for determining an immune response <i>in vitro</i> were well known. <i>See, e.g.</i> , WEIR'S HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (Leonore A. Herzenberg <i>et al.</i> eds., 5 <sup>th</sup> ed. 1996); and Sambrook <i>et al.</i> , MOLECULAR CLONING: A

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	LABORATORY MANUAL (1989), <i>supra</i> . Merely specifying that the immune response prior to optimization or following optimization be determined <i>in vitro</i> does not provide a patentable distinction over any of Claim 1, 3, or 5, each of which is anticipated by the Count. Accordingly, Claim 85 is obvious over the Count.
86. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined <i>in vivo</i> .	Claim 86 depends from any of Claims 1, 3, or 5, each of which is anticipated by the Count. Claim 86 merely adds that the immune response prior to optimization or following optimization is determined <i>in vivo</i> . Methods for measuring an immune response <i>in vivo</i> were well known. See, e.g., WEIR'S HANDBOOK OF EXPERIMENTAL IMMUNOLOGY (1996), <i>supra</i> ; Sambrook <i>et al.</i> , MOLECULAR CLONING: A LABORATORY MANUAL (1989), <i>supra</i> . Merely specifying that the immune response prior to optimization or following optimization be determined <i>in vivo</i> does not provide a patentable distinction over Claim 1, 3, or 5, each of which is anticipated by the Count. Thus, Claim 86 is obvious over the Count.

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**APPENDIX D**  
**COMPARISON OF THE '869 APPLICATION CLAIMS**  
**WITH THE PROPOSED COUNT**

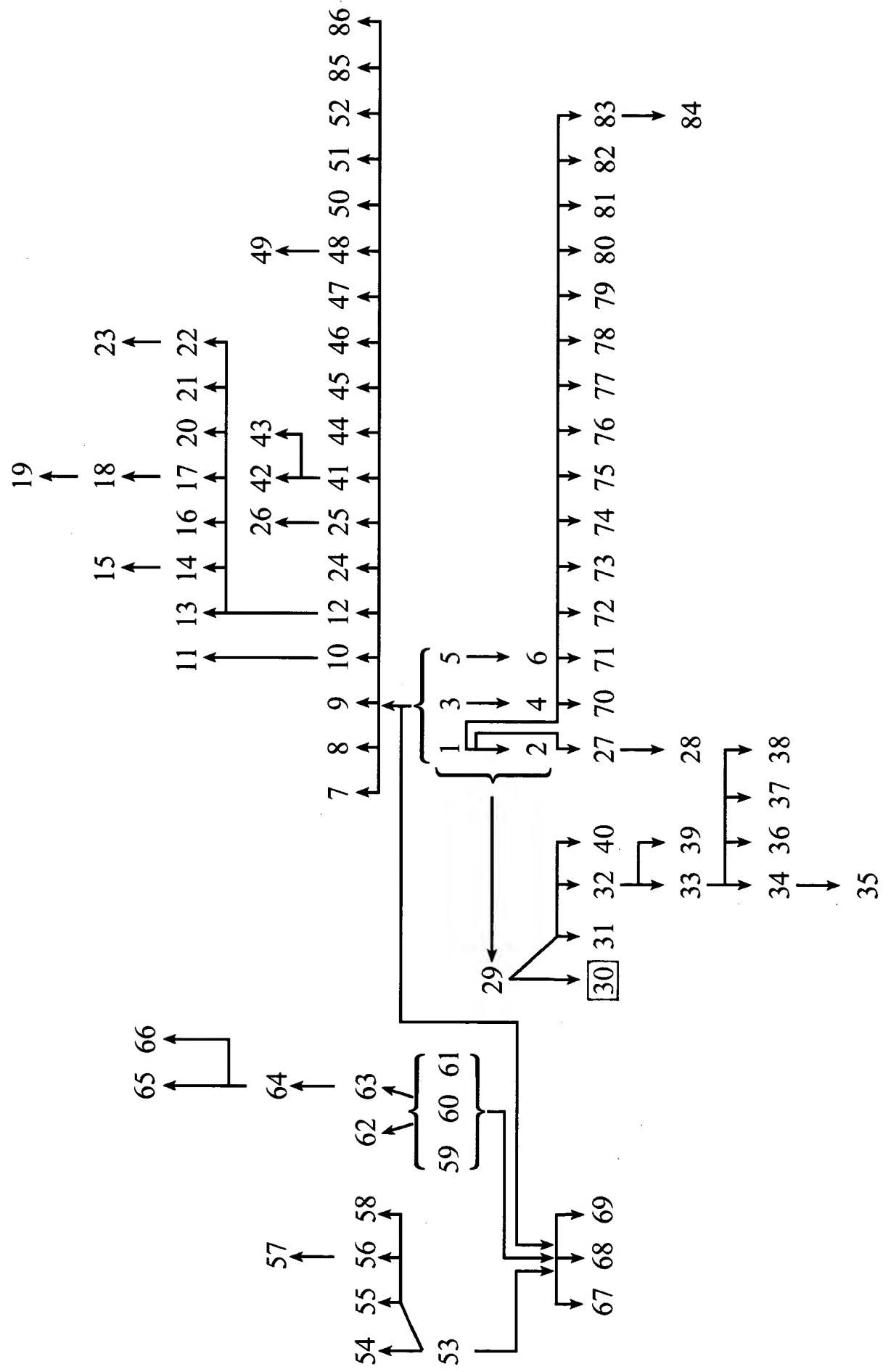
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<p>47. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising:</p> <p>a) creating a library of recombinant polynucleotides; and</p> <p>b) screening the library to identify an optimized recombinant polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector;</p> <p>wherein the optimized recombinant polynucleotide or the polypeptide encoded by the recombinant polynucleotide exhibits an enhanced ability to modulate an immune response compared to a polynucleotide from which the library was created;</p> <p>wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector,</p>	Claim 47 is one alternative of the Count. The limitations of Claim 47 include all the limitations recited in Claim 30 of the '258 patent, which depends from Claim 29, which depends in turn from any of Claims 1-6.

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'869 Application Claim	Claim Correspondence to the Proposed Count
<p>wherein the optimized recombinant polynucleotide encodes a co-stimulator selected from B7-1 (CD80) or B7-2 (CD86) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4,</p> <p>and whereby optimization is achieved by recursive sequence recombination.</p>	

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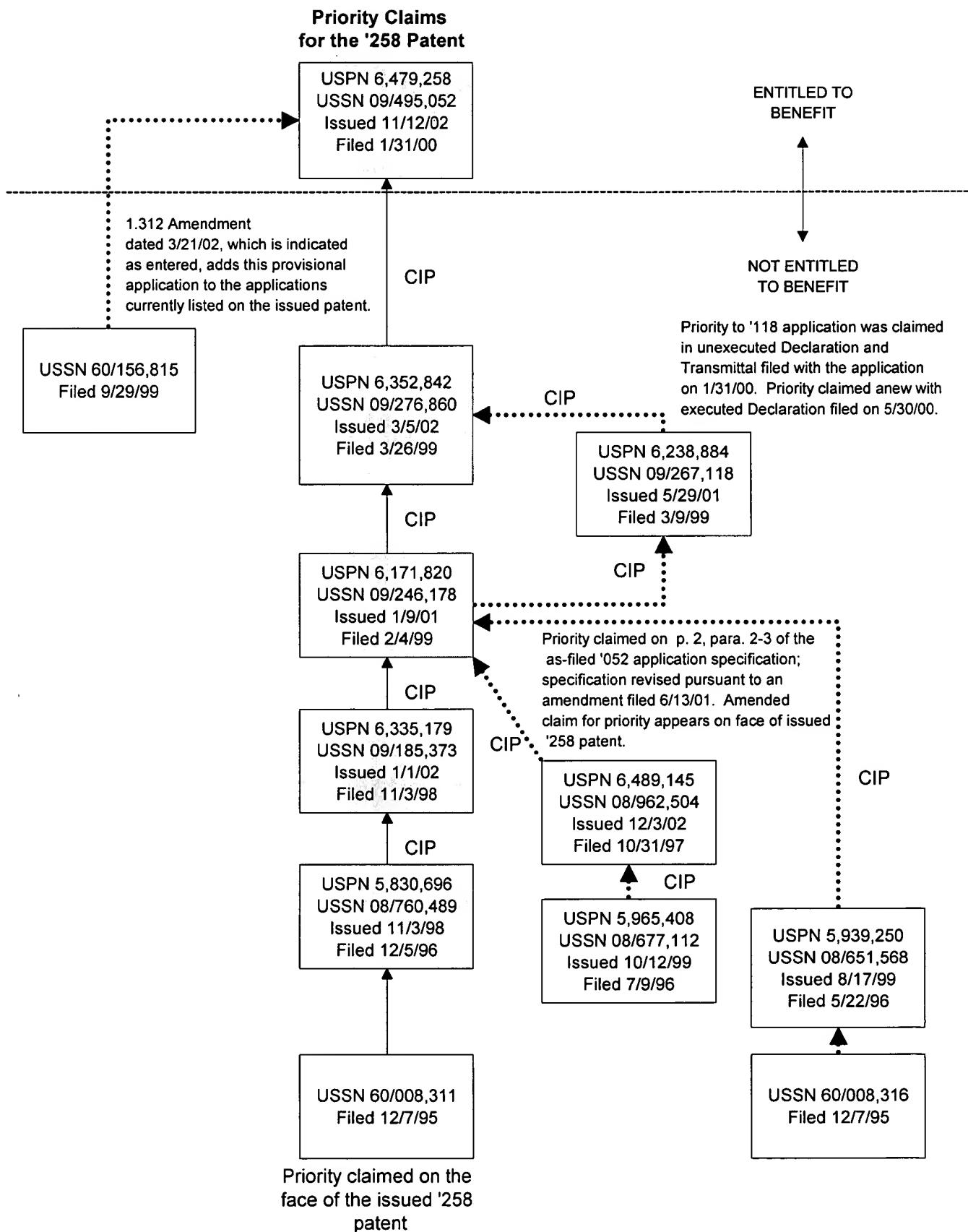
**APPENDIX E**



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**APPENDIX F**

APPENDIX F



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**APPENDIX G**  
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## Regioselective Enzymatic Acylation as a Tool for Producing Solution-Phase Combinatorial Libraries

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**Abstract:** A simple combinatorial strategy for sequential regioselective enzymatic acylation of multifunctional lead compounds has been developed and demonstrated using a polyhydroxylated flavonoid, bergenin, as a model. The approach is based on the ability of different enzymes to regioselectively acylate different sites on a lead molecule without affecting other similar functional groups. In sharp contrast to enzymatic acylation, conventional chemical acylation methods showed almost complete lack of regioselectivity. The enzymatic strategy was applied successfully to produce a solution phase combinatorial library of 167 distinct selectively acylated derivatives of bergenin on a robotic workstation in a 96-well plate format. General applicability of the automated combinatorial biocatalysis strategy is discussed. © 1998 Published by Elsevier Science Ltd. All rights reserved.

### INTRODUCTION

Biologically active lead molecules, either from natural isolates, rational design, or libraries from combinatorial synthesis typically contain multiple substituents that are chemically reactive. Because biological activity relies on the specific chemistry and arrangement of functional groups of a compound, the ability to derivatize some functionalities regioselectively, while leaving others free for alternative modifications and/or to interact with the biological target, can be important for drug development. Traditional chemical methods are generally not selective enough to allow derivatization of polyfunctional compounds without protection/deprotection schemes. The additional steps and purifications introduced to a library synthesis by these steps can be prohibitive, especially for solution phase combinatorial syntheses.

Enzymes are regioselective catalysts that have been used for the regioselective synthesis of pharmaceutically important compounds.<sup>1–8</sup> For instance, the regioselectivity of alcohol dehydrogenases allows the facile, selective reduction of dicarbonyl intermediates in the synthesis of the mycotoxin, zearalenone,<sup>1</sup> and the calcium channel blocker SQ31,765.<sup>2</sup> Likewise, oxygenases have been used extensively for the regioselective hydroxylation of many therapeutics, including important steroid derivatives,<sup>3</sup> and for the selective oxidation of one of two methyls to a carboxyl group in the synthesis of the antidiabetic agent, glipizide.<sup>4</sup>

Regioselective enzymatic acylations have also played an important role in the development of pharmaceutical compounds. For example, the HIV replication inhibitor, castanospermine, contains four chemically similar secondary hydroxyl groups. The chemical synthesis of selectively acylated derivatives, which are more potent than the parent molecule, requires a tedious, multi-step chemical procedure. In contrast, the bacterial protease subtilisin and lipase from *Chromobacterium viscosum* can acylate specific hydroxyls directly to produce the more active derivatives in a single step and high yield.<sup>5,6</sup> Regioselective acylations have also been used to produce the azole antifungal compound SCH 56592 for Phase II clinical studies,<sup>7</sup> and for the synthesis of a variety of acylated carbohydrates, nucleoside analogs, and steroids.<sup>8</sup>

The well-recognized regioselectivity of enzymes is ideal for solution phase combinatorial strategies with polyfunctional lead molecules. Moreover, enzyme and whole cell biocatalysts operate under uniform, mild reaction conditions; they therefore can be applied for a wide range of reaction chemistries with minimal side products and simple work-up before further reaction. Solution phase combinatorial strategies are particularly well suited for addressing the challenges of lead optimization. Solid phase syntheses would require development of a method for linking and cleaving the lead molecule and its derivatives to/from a solid support, thus limiting the locations and types of reactions that can be performed to generate a derivative library.

In the present paper, some of the unique advantages of regioselective combinatorial biocatalysis for solution phase synthesis of chemical libraries are illustrated through the preparation of a 167-compound library of derivatives of the polyfunctional natural flavonoid, bergenin. The synthetic scheme focuses on the use of enzymatic acylation to produce a derivative library of this flavonoid that could not be prepared using a chemical combinatorial scheme of similar simplicity. Thus, the regioselectivity inherent in combinatorial biocatalysis offers a powerful complement to chemical methods.

## RESULTS AND DISCUSSION

Bergenin contains five hydroxyl groups potentially amenable to enzymatic acylation (Fig. 1). To identify biocatalysts capable of regioselectively acylating specific sites on bergenin, we tested over fifty commercially available lipases and proteases as transesterification catalysts in organic solvents using vinyl

butyrate as a model acyl donor. Automation of the enzyme screen in 96-well polypropylene plates and high throughput mass spectrometric analysis (1 sample/min) allowed the rapid identification of several lipases that acylated bergenin in anhydrous CH<sub>3</sub>CN exclusively at the primary hydroxyl group (position 11 in Fig. 1) with nearly 100% conversion. The best enzymes included Chirazymes L-2 and L-9, and lipases PS30 and FAP-15. In subsequent experiments, a mixture of these lipases was used as a catalyst for regioselective acylation of the primary hydroxyl on the bergenin molecule. On the other hand, subtilisin Carlsberg suspended in a toluene-DMSO mixture (95 : 5 v/v) was found to produce a diacylated bergenin with substitutions at positions 4 and 11. Moreover, when bergenin-11-butyrat synthesized using the lipase catalyst was used as a substrate in subtilisin-catalyzed acylation with vinyl acetate, only position 4 was acylated.<sup>9</sup> Therefore, subtilisin can be used as a highly regioselective catalyst for 4-acylation of 11-substituted bergenin.

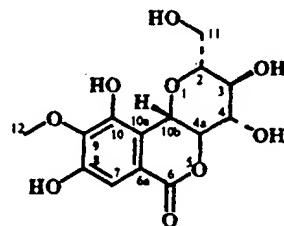


Fig. 1. Structure of bergenin

Based on these observations, the following general three-step synthetic strategy was suggested for generating a library including all possible 4,11- mono- and diacylated bergenin derivatives as discrete compounds (Fig. 2). In the first step bergenin is regioselectively acylated at the 11-position via lipase catalysis in dry CH<sub>3</sub>CN. When the reaction is complete, the immobilized biocatalyst is removed by filtration, and the 11-monoacylated bergenin derivative is recovered by evaporating the CH<sub>3</sub>CN. The clean product is then redissolved in a dry toluene-DMSO mixture, and added to immobilized subtilisin along with a second acyl donor. Because one of the positions on bergenin reactive to subtilisin-catalyzed acylation is already occupied (*viz.* position 11), the monoacylated product is selectively acylated at position 4, thus resulting in a homo- or hetero-4,11-diacylated bergenin derivative, depending on the acyl donor selected for the second step. Product can be recovered by filtering the solid enzyme, evaporating the solvent and extracting excess acyl donor. Finally, the regioselectivity of lipase for the 11-position can be used in the hydrolysis direction by replacing the reaction solvent with MeCN containing 2 % (v/v) water, to yield a selectively 4-monoacylated derivative. As in the previous steps, the product can be isolated by filtering the solid enzyme and evaporating the solvent. Using 96-well polypropylene filter-bottom reactors, as described in the Experimental Section, an entire library of N<sup>2</sup> + 2N derivatives can be made in parallel by applying this scheme combinatorially using N acyl donors.

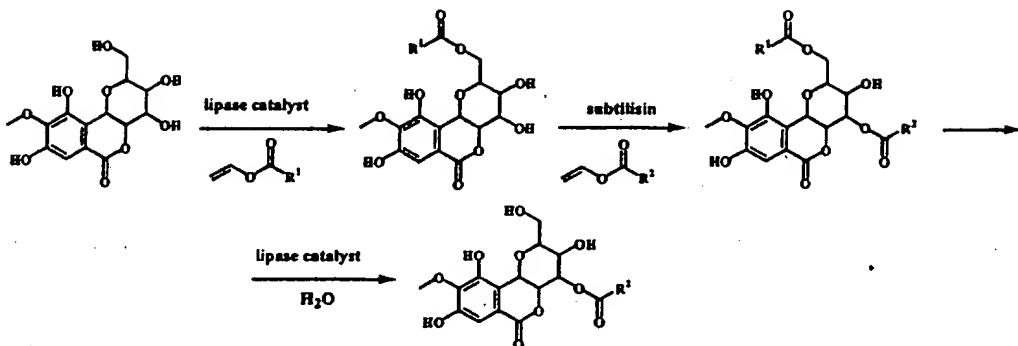


Fig. 2. Synthetic strategy for production of acylated bergenin derivatives by three-step regioselective enzymatic acylation/hydrolysis. The lipase catalyst was prepared by mixing equal weight parts of immobilized Chirazyme L-2, Chirazyme L-9, lipase PS30, and lipase FAP-15.

It should be noted that Fig. 2 describes a *general* strategy for generating the library of mono- and diacylated bergenin derivatives. Production of certain specific sublibraries can be accomplished by applying subsets of enzymatic acylations selected from this general scheme. For example, the sublibrary of 4,11-homodiacylated derivatives ( $R^1=R^2$ ) can be produced in one step by acylating bergenin with a single acyl donor using subtilisin as a catalyst. Similarly, the sublibrary of 4-monoacylated derivatives can be obtained from corresponding 4,11-homodiacylated derivatives by lipase-catalyzed regioselective hydrolysis (*cf.* the third step in Fig. 2). The choice of actual synthetic strategy is dictated by experimental convenience and whether the reactions are set-up and purified using automated or manual procedures.

The successful application of this synthetic strategy is illustrated by Fig. 3, which shows the results of HPLC and mass spectral analysis of the products obtained in a sequential acylation of bergenin according to the scheme in Fig. 2. In this example, bergenin (chromatogram in Fig. 3A) was first converted to a monoacylated derivative in a lipase-catalyzed reaction with vinyl butyrate (chromatogram in Fig. 3B). The product obtained in nearly quantitative yield after 96 h was further reacted with vinyl 2-methylthiophenecarbonate in the presence of subtilisin to give a heterodiacylated bergenin (80% yield in 96 h, chromatogram in Fig. 3C). To illustrate the regioselective hydrolysis (third step in Fig. 2), 4-butyrylbergenin was generated from 4,11-dibutyrylbergenin by selective enzymatic hydrolysis at the 11 position (85% yield in 96 h, chromatogram in Fig. 3D). Comparison of traces B and D in Fig. 3 clearly shows that the 11- and 4-monobutyrylated derivatives produced *via* lipase-catalyzed acylation of bergenin and selective hydrolysis of 4,11-diacylated bergenin (corresponding to the first and third steps in Fig. 2, respectively), have distinct retention times, but identical mass ions. Identities of all mono- and diacylated products were confirmed by HPLC/mass spectrometry.

The ability to selectively derivatize a single functional group on a multifunctional molecule is a unique feature of enzymatic catalysis that is typically very difficult to duplicate using conventional chemical methods. For example, when bergenin was chemically acylated with an equimolar amount of butyryl chloride, a mixture of several mono- and diacylated products was obtained, even at low conversions of bergenin (Fig. 3E). Similar results were obtained in acylation reactions of bergenin with benzoyl chloride or butyric acid/N,N'-dicyclohexylcarbodiimide. Clearly, in order to produce a library of selectively acylated bergenin derivatives using purely chemical methods, a complex synthetic scheme involving protection/deprotection steps would be needed.

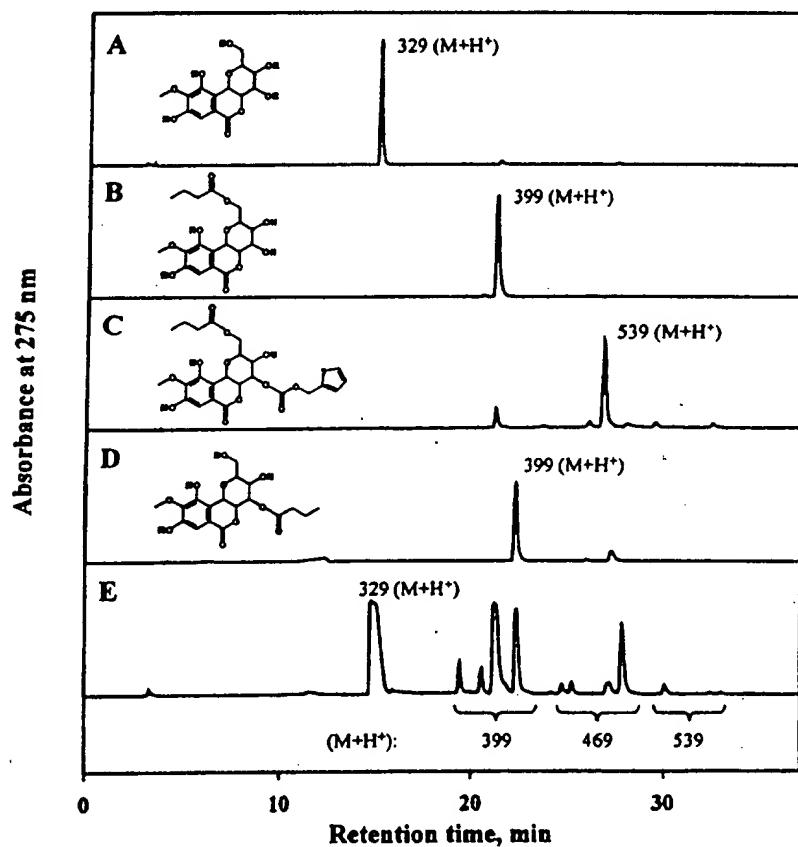


Fig. 3. Results of HPLC/MS analysis of the products of the three-step acylation/hydrolysis of bergenin. A. Starting bergenin. B. Product of acylation of bergenin with vinyl butyrate using the lipase catalyst. C. Product of acylation of the monoacylated butyrylbergenin with vinyl 2-methylthiophenecarbonate using subtilisin as a catalyst. D. Product of lipase-catalyzed hydrolysis of 4,11-dibutyrylbergenin. E. Product of chemical acylation of bergenin with butyryl chloride. Molecular weights 468 and 538 correspond to di- and triesters of bergenin and butyric acid, respectively.

The three-step enzymatic acylation/hydrolysis strategy was then applied to generate a library of mono- and diacylated bergenin derivatives. In this example, a set of twelve acyl donors of different types was used, including vinyl and trifluoroethyl esters and vinyl carbonates (Fig. 4). The acylations were performed combinatorially so that the twelve acyl donors were reacted with bergenin to produce all possible (24 monoacylated and 144 diacylated) derivatives as distinct/discrete compounds. All steps of the library synthesis, including reaction setup, sampling, and work-up, were performed using a robotic workstation as described in the Experimental Section. In order to achieve high conversions and increase the rate of enzymatic acylations, all acyl donors were used in 25-fold molar excess. Acyl donor remaining after completion of the first step was removed to prevent the formation of undesirable homodiacylated products in the second step. In most cases excessive acyl donors could be eliminated together with the reaction solvent by evaporation *in vacuo*. Less volatile donors were removed by extracting the dry residue obtained after solvent evaporation with hexane, which is a good solvent for the acyl donors but does not dissolve bergenin nor its derivatives.

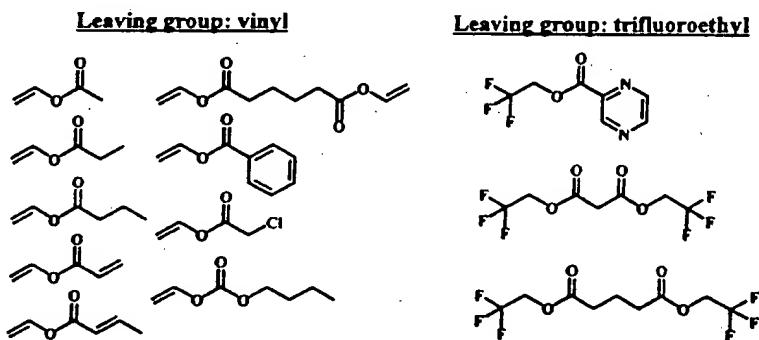


Fig. 4. Structures of acyl donors employed in the library synthesis. In the case of bifunctional acyl donors only one ester group of the acyl donor reacted, while the second was left intact.

Each of the bergenin derivatives synthesized using the three-step regioselective enzymatic acylation/hydrolysis was analyzed by HPLC/MS. In all cases except one the desired acylated products were found and showed the expected molecular mass in MS. The only unsuccessful derivative involved diacylation with two different bulky aromatic acyl donors (Fig. 4), which presumably impeded the second step acylation by creating steric hindrance at the active site of the enzyme. Based on HPLC data, overall yields of selectively acylated bergenin derivatives ranged from 60 to 90%.

In addition to the synthetic convenience afforded by regioselective enzymatic catalysis, the automation of library synthesis can be performed with inexpensive, readily available equipment and straightforward programming. A commercial robotic liquid handler, modified for operation with eight parallel septa-piercing

probes, was used to perform each step of library synthesis in commercially available 96-well plates. Reaction sampling and product recovery were simplified by the mild and non-corrosive solvents appropriate for biocatalysis, which allowed common septa materials to be used to seal the plate reactors. Biocatalysis also allows greater flexibility for the configuration of the synthetic process. In contrast to typical solid phase procedures for combinatorial synthesis, combinatorial biocatalysis can operate with either the substrate or immobilized catalyst in the solid phase. For instance, in the present work, immobilized lipases and subtilisin were used to selectively acylate bergenin, and the products were recovered in the solution phase by simple filtration from the catalysts through the bottom of the reactor. Since limited purification was required between the highly selective reactions, the necessary work-up could also be performed in 96-well plates on the robotic deck. The same simple equipment and procedures could be used for an iterative scheme involving a broad range of additional enzyme catalysts, which work under similar mild and uniform conditions for regiocontrolled synthesis.

The combinatorial biocatalysis approach described here is also unique in that it allows catalysts to be applied combinatorially to reaction development. In the present case, over fifty enzyme catalysts were screened in parallel as selective catalysts for the acylation of bergenin in a standard reaction, and 11 enzymes were identified as possible catalysts for the modification of bergenin. In this way, catalysts capable of acylating bergenin at different sites with various degrees of specificity were rapidly identified. Conceptually this process is very similar to high throughput screening for biological activity against enzyme targets, in which the reactions of a broad range of enzymes can be assayed using high density microplates and well established robotic liquid handlers. However, the development of automation for synthetic biocatalysis has not previously been described in the literature.

### Conclusion

As has been extensively demonstrated in nature, combinatorial biocatalysis exploits the inherent, proven strengths of enzymatic synthesis to produce small organic compounds of therapeutic value.<sup>10</sup> The biocatalytic process demonstrated herein can likewise be expanded to include enzymes that catalyze a broad range of organic reactions, such as hydroxylation, glycosylation, phosphorylation, halogenation, oxidations, and reductions, while retaining the advantages of solution phase synthesis.<sup>11,12</sup> Thus, combinatorial biocatalysis is capable of generating large derivative libraries, starting from a diversity of lead structures, to identify compounds with improved therapeutic potential.

Some advantages of this approach demonstrated by the acylated bergenin library include the opportunity for regioselective control over the compound library, mild and uniform reaction conditions, minimal side products, and simple work-up before further reaction. Practically, these advantages allow single-step derivatizations on a broad variety of lead molecules or scaffolds, including fragile or

chemically/stereochemically complex leads. Numerous enzymatic chemistries can be performed using simple and inexpensive automated synthesis, work-up, and analytical tools, thus allowing efficient parallel synthesis of pure compounds. These advantages provide a desirable alternative to combinatorial chemical synthesis techniques for the discovery of new lead structures possessing biological activity, and especially for the optimization of existing leads to identify possible clinical candidates.

Many developing technologies offer promise to enhance the utility of combinatorial biocatalysis. Revolutionary approaches for enzyme discovery, via PCR-based approaches such as shotgun cloning or panning with degenerate primers, has already greatly expanded the diversity of available biocatalysts.<sup>13</sup> Moreover, recent advances in protein overexpression<sup>14,15</sup> and affinity tag purification<sup>16,17</sup> offer the prospect of obtaining significant quantities of pure enzymes for practical application. Novel mutagenesis and gene shuffling strategies allow the production of customized synthetic enzyme catalysts<sup>18–21</sup> and new enzyme activities.<sup>22</sup> In addition, the development of combinatorial biosynthesis allows biocatalytic pathways to be utilized for combinatorial synthesis of “unnatural natural products” *in vivo* by altering the organic precursors or enzyme catalysts of the natural product pathways.<sup>23</sup> The increasing impact of these approaches, along with the growing importance of combinatorial chemistry, will continue to expand the capabilities of combinatorial biocatalysis.

## EXPERIMENTAL SECTION

### Enzymes

Chirazymes L-2 (lipase from *Candida antarctica*) and L-9 (lipase from *Mucor miehei*) were from Boehringer Mannheim (Indianapolis, IN). The polymer-supported enzymes were used without further modification. Lipases PS30 (from *Pseudomonas* sp.) and FAP-15 (from *Rhizopus oryzae*) were from Amano (Lombard, IL). Subtilisin Carlsberg (protease from *Bacillus licheniformis*) was purchased from Sigma (St-Louis, MO).

Lipases PS30 and FAP-15 were immobilized on Accurel polypropylene beads (Akzo Nobel, Obernburg, Germany, 200–400 µm particle size) following published procedures.<sup>24</sup> Subtilisin Carlsberg was lyophilized in the presence of 95% KCl by using the procedure of Khmelnitsky *et al.*<sup>25</sup> to increase the catalytic activity of the enzyme in the organic solvent.

### Solvents and Chemicals

All solvents were purchased from commercial suppliers and were of the highest purity available; they were stored over 3 Å molecular sieves (Linde). Analytical grade solvents for thin layer and flash column chromatography were used without further purification. Bergenin was purchased from Sigma, vinyl esters were all from TCI America. Other acyl donors were synthesized at EnzyMed according to the following procedures.

Glutaric acid di(trifluoroethyl ester). Glutaryl chloride (10 ml, 76 mmol) was added dropwise to trifluoroethanol (27 ml, 370 mmol) in 150 ml of dry  $\text{CH}_2\text{Cl}_2$ , containing 40 ml of pyridine, at 0°C under argon. Reaction mixture was stirred for 30 min at 0°C and then overnight at room temperature. The mixture was extracted with 2 M HCl and the organic layer was washed by sodium bicarbonate and water and dried with sodium sulfate. Dichloromethane was evaporated and the product was purified by vacuum distillation (b.p. 70–71 °C/1 Torr) to give 19 g (86%) of pure ester.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  2.02 (m, 2H), 2.52 (t,  $J=7.2$  Hz, 4H), 4.47 (q,  $J=8.6$  Hz, 4H).

2-Pyrazinecarboxylic acid trifluoroethyl ester. The mixture of 2-pyrazinecarboxylic acid (1.2 g, 9.6 mmol), N,N'-dicyclohexylcarbodiimide (2.1 g, 10.2 mmol), and  $\text{CF}_3\text{CH}_2\text{OH}$  (5 ml, 168 mmol) in 50 ml of dry dichloromethane was stirred overnight at room temperature. The reaction mixture was then filtered and the precipitate washed with ethyl acetate. The ethyl acetate and dichloromethane solutions were combined and evaporated. The residue was purified by flash chromatography on silica gel using ethyl acetate/hexane mixture (3 : 1 v/v) as eluent, to give 1.5 g (78%) of pale yellow solid ester.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  4.85 (q,  $J=8.6$  Hz, 2H), 8.81 (s, 1H), 8.84 (s, 1H), 9.35 (d,  $J=1.4$  Hz, 1H).

Malonic acid di(trifluoroethyl ester). Ditrifluoroethyl malonate was synthesized in the same manner as 2-pyrazinecarboxylic acid trifluoroethyl ester with 75% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.6 (s, 2H), 4.54 (q,  $J=8.6$  Hz, 4H).

Butyl vinyl carbonate was synthesized using a published procedure.<sup>26</sup>

#### *Acylation of Bergenin with Butyric Acid, Butyryl Chloride, and Benzoyl Chloride*

The mixtures of bergenin with equimolar amounts of butyryl chloride or benzoyl chloride, or butyric acid and N,N'-dicyclohexylcarbodiimide in dry pyridine were stirred overnight at room temperature. After solvent evaporation the dry residue was redissolved in methanol and analyzed by HPLC.

#### *General Procedure for Enzymatic Reactions*

All reactions were performed in 96-well (2 ml/well) glass-filled polypropylene filter plates (10  $\mu\text{m}$  polypropylene filter, Polyfiltronics, Rockland, MA). A custom sealing clamp and septa were used to allow sampling and prevent evaporation of the organic solvent during the reaction.

For the first acylation step, 15 mg of the immobilized lipase mixture (equal parts of PS30, FAP-15, Chirazyme L-2, and Chirazyme L-9) were added to each well in the plate. Using a Cyberlab C-200 liquid handler (Brookfield, CT), 25 mM of bergenin and 500 mM of the appropriate acyl donor in acetonitrile were added to the 96-well plate (final reaction volume 1 ml). The sealed 96-well plate reactor was then shaken (250 rpm) at 45 °C. Periodically, samples were automatically withdrawn and analyzed by HPLC and/or high-throughput MS (flow injection at ca. 1 sample/min). Upon completion of the first acylation reaction, the

enzyme was removed by filtration through the reactor bottom, the solvent removed under vacuum using a savant SpeedVac Plus centrifugal evaporator with a microplate rotor, and the excess acyl donor removed by washing (5x) with hexane.

The second acylation step was performed in the identical fashion as the first step. Subtilisin/95% KCl, 40 mg, was added to each well in the plate and toluene containing 5% (v/v) dimethyl sulfoxide was added for a total reaction volume of 1 ml; 2 mM of bergenin derivative and 50 mM of the appropriate acyl donor were used for the second step.

In the third hydrolysis step the same lipase mixture as in the first step above was used as a catalyst. The lipase mixture (50 mg) was added to 1 ml of 5–20 mM solution of 4,11-diacylated bergenin in MeCN containing 2 % (v/v) water. The reaction mixture was incubated under shaking (250 rpm) at 45°C for 96 h. Upon completion of the reaction, the solid enzyme was removed by centrifugation and the products were recovered by evaporating the solvent in vacuum.

#### *HPLC/MS Analysis*

Reverse phase HPLC analyses were performed on a Shimadzu SIL-10 HPLC (photodiode array detection) using an μBondapak C18 column (Waters, 3.9×300 mm) and a water/acetonitrile linear gradient program (100% to 20% water over 30 min with a 1 ml/min flow rate). HPLC/MS analyses were done using a Perkin-Elmer LC200 and a PE-Sciex API100 electrospray MS (turbo-ion spray head) with the same gradient program, a Phenomenex IB-SIL C18 column (2×100 mm), and a flow rate of 0.4 ml/min. The positive mode mass spectrum was collected along with the UV absorbance at 275 nm.

#### *NMR spectra*

NMR spectra were recorded in DMSO-d<sub>6</sub> with tetramethylsilane as internal standard on a Bruker WM-360 spectrometer, <sup>1</sup>H spectra at 360 MHz and <sup>13</sup>C spectra at 90 MHz. Spectral assignments were made by comparing the spectra obtained with the published spectra of bergenin and its acetylated derivatives.<sup>27,28</sup> Acylated bergenin derivatives were purified for NMR spectroscopy by flash chromatography on silica gel using chloroform/methanol mixture (15 : 1 v/v) as eluent.

Bergenin-11-butyrate: <sup>1</sup>H NMR: δ (ppm) 0.91 (t, J=7.5 Hz, 3H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 1.59 (q, J=7.2, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 2.34 (t, J=6.8 Hz, 2H, CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 3.32 (t, J=9.0 Hz, 1H, C3-H), 3.69 (t, J=10.0 Hz, 1H, C4-H), 3.79 (s, 3H, C12-CH<sub>3</sub>), 3.81 (m, 1H, C2-H), 4.01 (t, J=10.0 Hz, 1H, C4a-H), 4.13 (dd, J=11.8, 6.8 Hz, 1H, C11-H), 4.55 (d, J=11.8, 1H, C11-H), 5.02 (d, J=10.8 Hz, 1H, C10b-H), 5.65 (br.s., 1H, OH<sub>alifat</sub>), 5.73 (br.s., 1H, OH<sub>alifat</sub>), 7.03 (s, 1H, C7-H), 8.19 (s, 1H, OH<sub>arom</sub>), 9.80 (s, 1H, OH<sub>arom</sub>). <sup>13</sup>C NMR: δ 13.45 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 18.00 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 35.39 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O), 59.78 (C12), 63.15 (C11), 70.26 (C3), 72.24 (C10b), 73.49 (C4), 78.64 (C4a), 81.60 (C12), 109.70 (C7), 115.80 (C10a), 118.2 (C6a), 140.60 (C9), 148.60 (C10), 151.0 (C8), 163.40 (C6), 172.80 (CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>C=O). MS: 399 (M+H<sup>+</sup>).

**Bergenin-4,11-dibutyrate:**  $^1\text{H}$  NMR :  $\delta$  0.90 (t,  $J=7.4\text{ Hz}$ , 3H), 0.94 (t,  $J=7.3\text{ Hz}$ , 3H), 1.5 (m, 4H), 2.34 (t,  $J=7.2\text{ Hz}$ , 2H) 2.37 (t,  $J=7.2\text{ Hz}$ , 2H) - signals from two butyrates, 3.54 (m, 1H, C3-H), 3.77 (s, 3H, C12-CH<sub>3</sub>), 3.92 (m, 1H, C2-H), 4.16 (dd,  $J=10.8, 6.2\text{ Hz}$ , 1H, C11-H), 4.29 (t,  $J=9.8\text{ Hz}$ , 1H, C4a-H), 4.51 (dd,  $J=10.4, 1.4\text{ Hz}$ , 1H, C11-H), 5.16 (d,  $J=10.5\text{ Hz}$ , 1H, C10b-H), 5.30 (t,  $J=9.3\text{ Hz}$ , 1H, C4-H), 5.81 (d,  $J=6.5\text{ Hz}$ , 1H, OH<sub>alifat.</sub>), 6.99 (s, 1H, C7-H), 8.20 (s, 1H, OH<sub>arom.</sub>), 9.8 (s, 1H, OH<sub>arom.</sub>).  $^{13}\text{C}$  NMR:  $\delta$  13.31 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 13.37 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 17.87 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 18.03 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 35.25 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 35.49 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 59.75 (C12), 62.67 (C11), 67.92 (C3), 71.90 (C10b), 73.84 (C4), 76.67 (C4a), 78.21 (C12), 109.70 (C7), 115.40 (C10a), 118.0 (C6a), 140.70 (C9), 148.0 (C10), 151.0 (C8), 162.70 (C6), 172.20 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 172.70 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ). MS: 469 (M+H<sup>+</sup>).

**Bergenin-4-acetate-11-butyrate:**  $^1\text{H}$  NMR :  $\delta$  0.90 (t,  $J=7.4\text{ Hz}$ , 3H,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 1.57 (q,  $J=7.3\text{ Hz}$ , 2H,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 2.12 (s, 3H, OAc), 2.34 (t,  $J=7.2\text{ Hz}$ , 2H,  $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 3.56 (m, 1H, C3-H), 3.78 (s, 3H, C12-CH<sub>3</sub>), 3.91 (m, 1H, C2-H), 4.15 (dd,  $J=10.8, 6.2\text{ Hz}$ , 1H, C11-H), 4.29 (t,  $J=9.9\text{ Hz}$ , 1H, C4a-H), 4.50 (dd,  $J=10.4, 1.2\text{ Hz}$ , 1H, C11-H), 5.16 (d,  $J=10.5\text{ Hz}$ , 1H, C10b-H), 5.29 (t,  $J=9.3\text{ Hz}$ , 1H, C4-H), 5.87 (d,  $J=6.2\text{ Hz}$ , 1H, OH<sub>alifat.</sub>), 6.99 (s, 1H, C7-H), 8.23 (s, 1H, OH<sub>arom.</sub>), 9.84 (br. s, 1H, OH<sub>arom.</sub>).  $^{13}\text{C}$  NMR:  $\delta$  13.29 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 17.80 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 20.8 ( $\text{CH}_3\text{C}=\text{O}$ ), 35.15 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ), 59.67 (C12), 62.57 (C11), 67.83 (C3), 71.81 (C10b), 74.03 (C4), 76.50 (C4a), 78.02 (C12), 109.60 (C7), 115.40 (C10a), 117.90 (C6a), 140.70 (C9), 147.90 (C10), 150.90 (C8), 162.70 (C6), 169.60 ( $\text{CH}_3\text{C}=\text{O}$ ), 172.70 ( $\text{CH}_3\text{CH}_2\text{CH}_2\text{C}=\text{O}$ ). MS: 441 (M+H<sup>+</sup>).

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# FEE TRANSMITTAL for FY 2003

Effective 01/01/2003. Patent fees are subject to annual revision.

 Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT (\$ 180.00)

## Complete if Known

Application Number	099/724,869
Filing Date	November 28, 2000
First Named Inventor	Juha Punnonen
Examiner Name	Teresa Wessendorf
Art Unit	1639
Attorney Docket No.	0155.130US

## METHOD OF PAYMENT (check all that apply)

 Check  Credit card  Money Order  Other  None
 Deposit Account

Deposit Account Number	50-0990
Deposit Account Name	Maxygen, Inc.

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## FEE CALCULATION

## 1. BASIC FILING FEE

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 750	2001 375	Utility filing fee	
1002 330	2002 165	Design filing fee	
1003 520	2003 260	Plant filing fee	
1004 750	2004 375	Reissue filing fee	
1005 160	2005 80	Provisional filing fee	
SUBTOTAL (1)		(\$ 000.00)	

## 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Independent Claims	Extra Claims	Fee from below	Fee Paid
		-20** =	X	=
		- 3** =	X	=

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 84	2201 42	Independent claims in excess of 3
1203 280	2203 140	Multiple dependent claim, if not paid
1204 84	2204 42	** Reissue independent claims over original patent
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent
SUBTOTAL (2)		(\$ 0000.00)

\*\* or number previously paid, if greater. For Reissues, see above

## 3. ADDITIONAL FEES

Large Entity	Small Entity	Fee Description	Fee Paid
1051 130	2051 65	Surcharge - late filing fee or oath	
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130	Non-English specification	
1812 2,520	1812 2,520	For filing a request for ex parte reexamination	
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	
1251 110	2251 55	Extension for reply within first month	
1252 410	2252 205	Extension for reply within second month	
1253 930	2253 465	Extension for reply within third month	
1254 1,450	2254 725	Extension for reply within fourth month	
1255 1,970	2255 985	Extension for reply within fifth month	
1401 320	2401 160	Notice of Appeal	
1402 320	2402 160	Filing a brief in support of an appeal	
1403 280	2403 140	Request for oral hearing	
1451 1,510	1451 1,510	Petition to Institute a public use proceeding	
1452 110	2452 55	Petition to revive - unavoidable	
1453 1,300	2453 650	Petition to revive - unintentional	
1501 1,300	2501 650	Utility issue fee (or reissue)	
1502 470	2502 235	Design issue fee	
1503 630	2503 315	Plant issue fee	
1460 130	1460 130	Petitions to the Commissioner	
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	
1806 180	1806 180	Submission of Information Disclosure Stmt	180.00
8021 40	8021 40	Recording each patent assignment per property (times number of properties)	
1809 750	2809 375	Filing a submission after final rejection (37 CFR 1.129(a))	
1810 750	2810 375	For each additional invention to be examined (37 CFR 1.129(b))	
1801 750	2801 375	Request for Continued Examination (RCE)	
1802 900	1802 900	Request for expedited examination of a design application	

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Signature	<i>Margaret A. Powers</i>			Date	09-15-03

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# FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT	(S) 180.00
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## Complete if Known

Application Number	09/724,869
Filing Date	November 28, 2000
First Named Inventor	Juha Punnonen
Examiner Name	Teresa Wessendorf
Art Unit	1639
Attorney Docket No	0155.130US

## METHOD OF PAYMENT (check all that apply)

Check  Credit card  Money Order  Other  None

Deposit Account

Deposit Account Number	50-0990
Deposit Account Name	Maxygen, Inc.

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## FEE CALCULATION

### 1. BASIC FILING FEE

Large Entity Small Entity

Fee Code (\$)	Fee (\$)	Fee Description	Fee Paid
1001 770	2001 985	Utility filing fee	[REDACTED]
1002 340	2002 170	Design filing fee	[REDACTED]
1003 530	2003 265	Plant filing fee	[REDACTED]
1004 770	2004 385	Reissue filing fee	[REDACTED]
1005 160	2005 80	Provisional filing fee	[REDACTED]
<b>SUBTOTAL (1)</b>		(S) 000	

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

Total Claims	Independent Claims	Multiple Dependent	Extra claims	Fee from below	Fee Paid
xx	xx		-20" = xx	x 000 = 000	= 000
			- 3" = xx	x 000 = 000	= 000
				000 = 000	

Large Entity	Small Entity	Fee Description
1202 18	2202 9	Claims in excess of 20
1201 86	2201 43	Independent claims in excess of 3
1203 260	2203 145	Multiple dependent claim, if not paid
1204 88	2204 43	" Reissue independent claims over original patent
1205 18	2205 9	" Reissue claims in excess of 20 and over original patent
<b>SUBTOTAL (2)</b>		(S) 0000

\*or number previously paid, if greater. For Reissues, see above

## 3. ADDITIONAL FEES

Large Entity Small Entity

Fee Code (\$)	Fee (\$)	Fee Description	Fee Paid
1051 130	2051 65	Surcharge - late filing fee or oath	[REDACTED]
1052 50	2052 25	Surcharge - late provisional filing fee or cover sheet	[REDACTED]
1053 190	1053 190	Non-English specification	[REDACTED]
1812 2,520	1812 2,520	For filing a request for ex parte reexamination	[REDACTED]
1804 920*	1804 920*	Requesting publication of SIR prior to Examiner action	[REDACTED]
1805 1,840*	1805 1,840*	Requesting publication of SIR after Examiner action	[REDACTED]
1251 110	2251 55	Extension for reply within first month	[REDACTED]
1252 420	2252 210	Extension for reply within second month	[REDACTED]
1253 950	2253 475	Extension for reply within third month	[REDACTED]
1254 1,480	2254 740	Extension for reply within fourth month	[REDACTED]
1255 2,010	2255 1,005	Extension for reply within fifth month	[REDACTED]
1401 330	2401 165	Notice of Appeal	[REDACTED]
1402 330	2402 165	Filing a brief in support of an appeal	[REDACTED]
1403 290	2403 145	Request for oral hearing	[REDACTED]
1451 1,510	1451 1,510	Petition to institute a public use proceeding	[REDACTED]
1452 110	2452 55	Petition to revive - unavoidable	[REDACTED]
1453 1,330	2453 665	Petition to revive - unintentional	[REDACTED]
1501 1,330	2501 665	Utility issue fee (or reissue)	[REDACTED]
1502 480	2502 240	Design issue fee	[REDACTED]
1503 640	2503 320	Plant issue fee	[REDACTED]
1460 130	1460 130	Petitions to the Commissioner	[REDACTED]
1807 50	1807 50	Processing fee under 37 CFR 1.17(q)	[REDACTED]
1806 180	1806 180	Submission of Information Disclosure Stmt	180.00
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1809 770	2809 385	Filing a submission after final rejection (37 CFR 1.129(a))	[REDACTED]
1810 770	2810 985	For each additional invention to be examined (37 CFR 1.129(b))	[REDACTED]
1801 770	2801 985	Request for Continued Examination (RCE)	[REDACTED]
1802 900	1802 900	Request for expedited examination of a design application	[REDACTED]

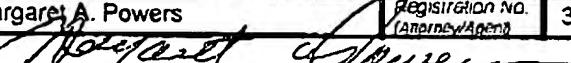
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